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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1975

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FOR THE COMMANDER

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The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1974 through May 1975 are reviewed in this report. Acute toxicity experiments were conducted on SYFO, FEFO, hydrazine, MMH and UDMH. Chronic toxicity experiments were conducted with MMH in drinking water, coal tar aerosols, inhaled UDMH and with a mixture of dichloro-methane and 1,1,1-trichloroethane. Oral and percutaneous toxicity determinations and skin irritation and skin sensitization studies were made on a

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number of transportable chemical agents.

PREFACE

This is the twelfth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contrat No. F33615-73-C-4059. This document constitutes the third and final report under the current contract and describes the accomplishments of the THRU from June 1974 through May 1975.

The current contract for operation of the Laboratory was initiated in 1972 under Project 6302 "Toxic Hazards of Propellants and Materials," Task 01 "Toxicology" Work Unit No. 63020113. K. C. Back, Ph. D., Chief of the Toxicology Branch was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph. D., served as co-principal investigator and Laboratory Director for the THRU of the University of California, Irvine. Acknowledgement is made to C. E. Johnson, C. C. Haun and G. L. Fogle for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the National Institute of Occupational Safety and Health, the National Aeronautics and Space Administration and the Department of Transportation.

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SECTION I

INTRODUCTION

This document constitutes the 12th annual report of the Toxic Hazards Research Unit, (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the Air Force and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories. The chamber facilities consist of two types, each performing a separate function. Rochester and Longley Chambers are used for exposing animals to airborne contaminants under ambient conditions of pressure and air composition. These ambient chambers are useful for acute inhalation exposures as well as intermittent long-term chronic exposure experiments. Eight unique and extremely versatile altitude chambers (designated herein as Thomas Domes) are used for conducting long term continuous or intermittent subacute and chronic exposure studies. These Thomas Domes are capable of operating at absolute pressures ranging from 260 to 760

torr utilizing gas mixtures ranging from 20 to 100% oxygen and 0-80% of a secondary gas or mixture of gases. Environmental control of relative humidity, temperature, pressure, and gas flow rate is very stable and precise through continuous monitoring and feedback modulation of regulating valves. The control equipment is provided in replicate and failsafe form so that uninterrupted exposures may be conducted for indefinite periods. More detailed description of the design and operation of the THRU facility is published (Fairchild, 1967; MacEwen, 1965; MacEwen and Geckler, 1966; MacEwen and Vernot, 1968, 1969, 1970; Thomas, 1968).

With the comprehensive scientific team and exposure resources described above the THRU can conduct realistic simulation of human exposures to contaminants causing adverse health effects. These exposures, provided to multiple animal species, are carefully monitored using continuous analytical techniques. The animals used in the experimental programs are also monitored by continuing visual observation and regularly scheduled biochemical and physiological measurements.

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazard of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineer-

engineering design factors. This research effort is continuing on a lesser scale while more emphasis has been placed on obtaining data useful for solution of problems of military or civil aircraft emergencies, community emergencies, and chronic industrial exposures. To this end many of the current research programs serve the mutual interest of the Air Force and other governmental agencies such as the National Institute of Occupational Safety and Health, and the Department of Transportation.

As part of its contract responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to Air Force, other governmental and industrial scientists. This year's conference chaired by Dr. B. D. Culver presented 27 technical papers and had as a central theme environmental carcinogenesis. Other sessions were concerned with environmental quality and cellular toxicology. Six papers were presented by University of California faculty and staff members. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 24 September through 26 September, 1975, drew 154 participants including speakers.

The papers presented at the conference were published as the Proceedings of the 5th Annual Conference on Environmental Toxicology, AMRL-TR-74-125, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio (415 pages).

Next year's conference, currently in the development stage, will be held in October, 1975 at the Biltmore Towers Hotel in Dayton, Ohio.

SECTION II

RESEARCH PROGRAM

The research activity of the THRU is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broad range of chemical materials and includes inhalation studies of aircraft fuels, coal tar aerosols, halogenated hydrocarbons, rocket fuels and combination of solid rocket propellant exhaust products. Acute oral and dermal toxicity studies on transportable materials were also conducted.

A 6-Month Chronic Inhalation Exposure of Animals to UDMH to Determine Its Oncogenic Capacity

Hydrazine, unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) have each been shown to produce carcinomas in experimental animals by various oral modes of administration (IARC, 1974; Clark et al., 1968). Although this information about the hydrazines and many other chemical compounds has scientific interest,

it is frequently unrealistic in terms of actual or practical human exposures. Recently, however, experiments in the Toxic Hazards Research Unit Laboratory of UC, Irvine have confirmed the carcinogenic risk of the Threshold Limit Values (TLV) of 1 ppm hydrazine (MacEwen and Vernot, 1974). In these experiments, mice held 1 year postexposure after 6 hours daily, 5 days per week inhalation exposure to N_2H_4 over a 6-month period were necropsied and found to have a significant increase in alveolargenic carcinomas at the industrial TLV concentration. At a 5-fold higher dose, 11 of 15 exposed mice had alveolargenic carcinoma, 2 had lymphosarcomas and 1 mouse had a malignant hepatoma with metastasis of the spleen. Two of the mice with alveolargenic carcinomas also had metastatic lesions, one in the heart and another in the rib cage.

The results of these studies indicate a strong dose relationship for the production of carcinomas in mice. A dose relationship was seen for other inhalation effects of hydrazine and MMH, either acute or chronic (Haun, 1970; MacEwen and Haun, 1971).

The evidence that N_2H_4 produces pulmonary carcinomas at the TLV concentration is based on small numbers of animals in only one species and should be confirmed in multiple species and with large numbers of animals.

There is a question as to the likelihood that human exposures of this chronic nature have occurred during the production and use of hydrazine, UDMH and MMH. Moreover, there is no certainty that chemicals that produce tumors in mice will be carcinogenic for man. In spite of these reservations but cognizant of the fact that the hydrazines do cause carcinogenic reaction in animals, it was desired that inhalation studies be performed by THRU which will be definitive of a suitable carcinogenic risk or no effect level for these highly important industrial and military chemicals.

A careful review of carcinogenic testing methods was conducted to define what animal species and numbers should be used. Carcinogenic screening procedures generally utilize 100 animals of each species to be tested. To achieve our goal, it was decided that groups of 400 mice, 200 rats and hamsters, and 8 dogs will be exposed to 3 air concentrations each of UDMH, hydrazine and MMH at or near their respective TLV's. Exposures will be on a daily industrial exposure basis for 6 months followed by a suitable period for cancer induction in each species. The numbers of animals are based on the maximum numbers for each species which can be exposed in the THRU chambers and were selected to permit a statistically valid number of animals of each species to reach the required age for tumor induction with natural and toxicologic attrition. One large

animal species (the dog) was selected because it is the most sensitive animal to other chronic effects of the hydrazines (hemolytic and CNS) and is also the most suitable species for monitoring hematologic and biochemical status during the experimental period. The long term postexposure holding of the dogs with periodic testing of hepatic function will also provide the data base for determining the capability of the individual hydrazines to produce delayed liver damage.

Although N_2H_4 has the highest scientific priority, Air Force needs dictate that UDMH studies be undertaken first to be followed immediately by N_2H_4 studies and finally MMH.

Reported here are the results of completed 6-month exposures of animals to 5 ppm and 0.5 ppm UDMH. Utilization of Thomas Domes for other studies disallowed the testing of the 0.05 ppm level of UDMH simultaneously. However, information collected through 4 months of 0.05 ppm exposure will be presented.

Animals used in this study, in the numbers mentioned previously, consisted of female C57 black/6 mice obtained from Jackson Laboratories, male CDF (Fischer 344 derived) albino rats from Charles River, male Engle Golden Syrian hamsters, and beagle dogs, 4 male and 4 female per group. A separate set of control animals were provided for the

0.05 ppm test since it was not started at the same time as 5 ppm and 0.5 ppm experiments. Two chambers were used for each UDMH air concentration. Each pair of chambers contained as few species as possible to minimize risk of cross infection. Dogs and rats were housed in one dome and mice and hamsters in the companion chamber. All control animals were maintained in animal holding facilities.

The Thomas Domes were operated with nominal airflows of 35 CFM at a slightly reduced pressure of 725 mm Hg to prevent leakage of UDMH into the laboratory. Exposures were conducted on a 6 hour/day, 5 day/week schedule. No exposures were made on weekends and holidays.

The control of potential carcinogens in cancer research laboratories is essential for the prevention of occupationally acquired cancer and for the protection of the general environment from exposure to potential cancer inducing materials. Since the consequences of laboratory exposures to experimental carcinogens may not be demonstrated for many years, preventive measures, National Cancer Institute Safety Guidelines were followed in the conduct of this study and will be adhered to in the subsequent testing of hydrazine and MMH. To this end then, special memoranda were posted in the laboratory and warning signs were placed at all access doors. The exposure laboratory was off

limits to visitors and staff personnel not actually involved in the conduct of the study. The tasks of the technicians involved entries into the chambers following the completion of the daily exposures. Protective clothing used were coveralls, hoods, gloves, and boot covers. All of this equipment is disposable paper or plastic and was discarded daily in sealed plastic bags. Air supplied full face masks were used for respiratory protection. All technicians showered after the last dome entry. Analytical measurements made by the THRU Chemistry Department provided assurance that UDMH concentrations were extremely low or nonexistent in the UDMH generation hoods, in the laboratory spaces adjacent to the chambers, and in the effluent air and vacuum pump water. Analytic details and results may be found in the Analytical Chemistry Programs section of this annual report.

The chamber concentrations of UDMH were generated and continuously monitored with apparatus and instrumentation essentially the same as used for chronic hydrazine and MMH studies in the past. Details may be found in previous technical reports (Haun, 1970; MacEwen and Haun, 1971) and this 1975 report.

All test animals were observed hourly during exposure and non-exposure periods for signs of UDMH intoxication and mortality. Gross and histopathologic examination was made on all dead animals. Rats, hamsters and dogs were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups and group mean weights followed on a monthly basis throughout the experimental period. Blood samples were drawn from dogs at biweekly intervals and clinical determinations made for the following battery of tests:

RBC	Sodium	Albumin
WBC	Potassium	Globulin
HCT	Calcium	SGPT
HGB	Glucose	Alkaline Phosphatase
Differential Cell Count	Total Protein	

Blood measurements not included in regular biweekly schedule during the exposure phase of the study but made at the conclusion of the 5 ppm and 0.5 ppm experiments were:

Blood urea nitrogen	SGOT
Chloride	Prothrombin time
Cholesterol	Cephalin flocculation
Creatinine	Bromsulphalein.

Of these, tests giving abnormal values were scheduled to be repeated postexposure at regular intervals until recovery. To examine for possible hemolytic effects in rodents, blood samples for hematocrit and red blood cell counts were taken from 5 rats and 5 hamsters from each group at the conclusion of the 5 ppm and 0.5 ppm exposures. Blood was withdrawn using a nondestructive suborbital technique.

Overt signs of UDMH toxicity were nonexistent in animals exposed for 6 months to 5 ppm and 0.5 ppm UDMH. Growth curves for both groups of dogs and mice were normal when compared to those of the control groups. In the case of the rats, however, statistically significant lower weight-gain rates were noted for both exposed groups throughout the 6 months of exposure. Hamster weights were somewhat erratic and showed no weight gain when mean weights at exposure conclusion were compared with initial weights. Their mean weights with one exception (the 5 ppm exposed group at 4 weeks of exposure) were significantly lower than control during the entire exposure period. Effects on weight of exposed rats or hamsters were not dose dependent.

The numbers of animals that died during the 6 months of exposure to 5 ppm and 0.5 ppm UDMH are shown in Table 1.

TABLE 1. MORTALITY RATIOS IN CONTROL AND UDMH EXPOSED ANIMALS

Experimental Group	Dogs	Rats	Mice	Hamsters
Control	0/8	0/200	7/400	13/200
0.5 ppm	0/8	0/200	6/400	24/200
5 ppm	0/8	1/200	8/400	22/200

The mortality figures for exposed hamsters can be misleading. In no case was death attributed to UDMH exposure. Deaths were due to pneumonia, and particularly injuries from manipulation of cage catch pans during cleaning operations early in the study. This problem was eliminated by relocation of the hamster cages where catch pans were not required. Death in groups of exposed and control mice were approximately the same; therefore, no toxicological significance is attached to mortality in exposed mice.

Examination of hematocrit and RBC determinations made immediately postexposure on rats and hamsters showed no abnormalities. Likewise, results of hematocrit, hemoglobin, RBC and reticulocyte measurements on dogs showed no effects of exposure to UDMH.

Results of clinical chemistry tests made on dogs biweekly during and following exposure were all normal except for serum glutamic pyruvic transaminase (SGPT) values shown in Table 2. It can be seen that SGPT values were significantly elevated in dogs exposed to 5.0 ppm UDMH after the first biweekly sampling period. A sharp reduction, approximately 50%, occurred at 2 weeks postexposure. Recovery, however, was not complete by 4 weeks postexposure where the SGPT value remained significantly elevated above control and nearly the same as the 2 week postexposure result. In many cases, dogs exposed to 0.5 ppm UDMH had slightly higher values than controls; however, this was true before exposure and at the first sampling period indicating that the normal SGPT mean in this group was slightly higher than that of the controls.

Special liver function tests were performed on dogs at exposure termination. Prothrombin time and cephalin flocculation values were normal but bromsulphalein (BSP) measured in blood of the 5 ppm exposed dogs 10 minutes following 10 mg/kilo injection showed significant retention. This test was repeated at 4 weeks postexposure with similar results. All BSP values are shown in Table 3.

TABLE 2. MEAN SGPT VALUES[1] FOR GROUPS OF 8 BEAGLE DOGS EXPOSED FOR 6 MONTHS TO UDMH

Weeks of Exposure	Control	0.5 ppm	5 ppm
2	25.8	35.3*	32.4
4	26.8	34.5*	78.6**
6	26.9	32.8	102.4**
8	24.5	37.0*	118.0**
10	26.4	32.0**	118.0**
12	30.8	33.3	115.5**
14	--	--	--
16	22.0	33.4**	87.8**
18	22.8	26.6*	106.5**
20	22.5	25.6	99.3**
22	19.8	28.1**	97.0**
24	21.5	31.0*	100.3**
26	24.5	26.5	86.3**
Weeks Postexposure			
2	22.1	24.5	36.6**
4	22.5	27.6*	41.6*

[1]International Units

* Significant at the 0.05 level.

**Significant at the 0.01 level.

TABLE 3. MEAN BROMSULPHALEIN RETENTION VALUES* IN CONTROL AND UDMH EXPOSED DOGS

Time	Control	0.5 ppm	5 ppm
Exposure Termination (26 Weeks)	18.1	18.5	30.3**
Postexposure (4 Weeks)	20.7	16.8	29.5**

* Percent retention
** Significantly higher than controls at the 0.05 level.

Although the mean BSP retention values for the 5 ppm exposed dogs at 4 weeks postexposure indicate no trend to recovery, an examination of individual values revealed 10-25% reductions in values for 6 of 8 dogs.

Four of the planned 6 months of animal exposure to 0.05 ppm UDMH have been completed. Hepatotoxicity as measured by SGPT serum levels has not been demonstrated in the 0.05 ppm dog exposure group. All clinical measurements are normal and show no trends to adverse effect. Mean body weights of dogs and mice are comparable with controls while weights of rats and hamsters are approximately 3% less than their control groups. These differences are statistically but not biologically significant. Fifteen of 400 have died, but all deaths were unrelated to UDMH exposure.

The desired daily UDMH concentration levels of 5 ppm and 0.5 ppm were maintained with good precision during the entire six months of the study.

Significant exposure effects of UDMH were limited to slight to moderate hepatotoxicity in dogs exposed to the 5 ppm concentration with indications of partial recovery at 4 weeks postexposure. The absence of toxic effects in the 6 month 0.5 ppm exposed animals implies that the 0.05 ppm level of UDMH will also prove to be nontoxic at completion of the exposure period. On the basis of results of tests and measurements used in this study, the current industrial TLV of 0.5 ppm UDMH appears to be well chosen without consideration of cancer risk. Cancer incidence will be assessed during the lifetime observation and testing of the rodents and dogs.

Continuous Inhalation Exposure of Rodents to Perfluoropentane for 14 Days

Flutec - FC50 is primarily perfluoropentane, which is a candidate material for use as a heat exchanger medium in the air handling system of spacecraft. Perfluoropentane has a molecular weight of 288, density of 1.6 g/ml and a boiling point of 29 C. The Toxic Hazards Research Unit of UCI was requested to determine the acute toxicity hazard of a 5% vapor concentration of Flutec for rats and mice. Because Flutec is very expensive and only a small quantity was provided for testing, two closed loop recirculating life support systems were used in the test program. These recirculating chambers were similar in design to chambers used previously for toxicity screening of space cabin materials for NASA.

Groups of 100 female CF-1 mice and 30 male CFE rats were exposed for periods up to 14 days to 5% (50,000 ppm) perfluoropentane (C_5F_{12}) in a closed loop system. Control mice were housed in hanging cages located around the periphery of the exposure chamber as well as in a loop. Rat controls were caged only within a recirculating chamber. Thirty rats or 50 mice occupied a single chamber.

All chambers were continuously monitored for CO_2 and O_2 concentrations and temperature. Oxygen was bled into each closed loop system to replace the amount used by the experimental animals for metabolism. An alarm system was used to indicate any excursion in CO_2 or drop in O_2 content beyond predetermined limits. Lithium hydroxide canisters were used to remove metabolically produced CO_2 from the loop system environment. Temperature and relative humidity measurement were recorded hourly. The exposure chambers were maintained at a slight negative pressure to avoid outward leaks of the test material.

Liquid Flutec was injected and vaporized into each test loop in precalculated quantities, to maintain the desired exposure concentration. Preliminary runs with the loop systems determined the frequency of supplemental injections needed to maintain a stable 5% Flutec concentration.

Analysis of contaminant concentration was made with a Varian A-90 gas chromatograph (thermal conductivity detector) using a Porapak Q column. Automatic sequential samples of the atmosphere in each of the test loops were taken once hourly for chamber monitoring.

Animals were observed daily for appearance, behavior, signs of toxic stress and lethality with particular emphasis on the possible anesthetic effect of the test compound.

Rat body weights, test and control, were obtained immediately before and at the conclusion of the exposure period. A complete histopathological examination of major organ systems including brain, lung, liver, kidney, spleen and heart was performed on groups of 15 test and 15 control rats at completion of the exposure and at two weeks postexposure. Organ and body weights were obtained at these sacrifices for organ/body weight calculations.

Except in one case, mortality in exposed mice prevented the planned serial sacrifice for liver triglyceride measurements on days 1, 3, 7, 10 and 14 during exposure and on days 1, 3, 7 and 14 days postexposure. Animal body and liver weights were to be obtained at the same intervals for liver to body weight calculations. Pathology information was obtained on mice that died and on one group that survived the exposure period and were sacrificed 14 days postexposure.

There were no rat deaths during exposure, and all survived the 14-day postexposure observation period.

Table 4 lists the mean body weights of rats measured at various times during the experiment and Table 5 the mean organ weights obtained immediately postexposure. Continuous exposure to a 5% air concentration of Flutec for 14 days led to significant increases in all organ weights except the spleen even though rat growth was significantly depressed during exposure. Gross pathological examination revealed mottled kidneys in 12 of the 15 rats examined, lung hemorrhage in 3 and liver congestion in 4. Pulmonary changes seen in most rats were inflammatory in nature.

Exposures of 100 mice to 5% Flutec under the same conditions as the rats led to considerable mortality. Thirty-three of the 50 mice exposed in one loop died between the third and fourth day of exposure. Five of the group had been removed on exposure days one and three for liver triglyceride measurements. Therefore, the final mortality ratio was 33 of 40. This portion of the experiment was terminated. The exposure of 50 mice in a second loop was continued and completed the 14-day period without mortality. Groups of 10 each were sacrificed at 1, 3, 7 and 14 days postexposure for body and liver weights, liver triglyceride determination and pathologic examination. Of this information, only the body and liver weight information is available but statistical treatment has not been made.

TABLE 4. EFFECT OF CONTINUOUS INHALATION EXPOSURE TO 5% PERFLUOROPENTANE ON RAT BODY WEIGHTS(1)

Exposure Time	Exposed, g	Chamber Control, g
1 Day Preexposure	226.5	224.4
0 Day	229.7*	225.2
4 Days	216.6**	237.9
7 Days	236.2**	245.6
11 Days	245.3**	260.1
13 Days	249.1**	266.3
3 Days Postexposure	277.9	286.7
7 Days Postexposure	298.5	306.5
10 Days Postexposure	308.3	314.7
13 Days Postexposure	322.1	328.8

(1) N = 30
* Different from control mean at 0.05 significance level
** Different from control at 0.01 significance level

TABLE 5. EFFECT OF 14-DAY CONTINUOUS INHALATION EXPOSURE TO 5% PERFLUOROPENTANE ON RAT ORGAN WEIGHTS(1)

Organ	Exposed, g	Chamber Control, g
Heart	1.22*	0.98
Lung	1.79*	1.46
Liver	10.98*	9.44
Spleen	0.78	0.78
Kidney	2.43**	2.23

(1) N = 15
* Different from control mean at 0.01 significance level
** Different from control mean at 0.05 significance level

After a thorough recheck of all environmental control parameters, another group of 50 mice were exposed in the first loop in an effort to resolve the obvious conflicting results in the previous experiment. By 3 days of exposure, 12 of 50 mice were dead and the experiment was terminated. The second loop which had been the control chamber in the second test run was then used to expose a separate set of mice to 5% Flutec. The experiment was concluded after 3 days of exposure when 29 of 30 mice had died. This loop was tested again, without Flutec and 30 mice survived 10 days in the chamber with no ill effects.

Examination of the mice that died in each of the test runs was consistent with a diagnosis of chemical pneumonitis. This diagnosis is compatible with the changes seen in the rats exposed to the same concentration of Flutec and with the measured rat organ weight changes.

The experimental results of the rat and mouse data suggested a number of possible reasons for the observed edemagenic effects and animal deaths (mouse). Flutec could be a pulmonary irritant at the 5% concentration used. Possibilities also exist that impurities in the agent or chemical breakdown of the agent or its impurities may be the causative agent(s). It occurred to us that the Flutec might be chemically changed by repeated passage through the lithium hydroxide used to scrub the system of CO_2. To test this possibility 6 mice were exposed

in a dynamic flow system to Flutec which did not pass through lithium hydroxide and was not recirculated while 6 mice were exposed to the agent after passing once through lithium hydroxide and was not recirculated while 6 mice were exposed to the agent after passing once through lithium hydroxide. Of course, we realized that any toxic material created by one passage through lithium hydroxide might be present in sufficient concentrations to cause toxic effects. The concentration was kept at approximately 5% for the first seven days after which it was raised to 7.5%. Pulmonary edema was not evident and the animals showed no effects such as were found in the closed loop system. These data tend to negate the possibility that Flutec per se is the causative agent. If it were, one would expect there to be increased mortality with increased concentration and further it would appear that production of a toxic material must take place during the recycling procedure.

In an effort to detect and identify possible toxic materials produced under the experimental conditions, a 5% concentration of Flutec was recirculated through the exposure chamber without animals for a 2-week period and samples taken for gas chromatographic-mass spectrometric analysis by 6570 AMRL/THE and NASA. No report of the outcome of these analysis has yet been made.

Chronic Inhalation Toxicity of JP-4 Jet Fuel

An 8-month intermittent study on the toxicity of JP-4 jet fuel to various animal species was detailed in the previous annual report (MacEwen and Vernot, 1974). The study was terminated after 33 weeks of exposure. With the exception of 20 rats and 20 mice to be held one year postexposure, all animals were sacrificed.

Gross pathological examination of the monkeys, rats and mice revealed no lesions which could be attributed to exposure. Organ and body weight ratios for rats have been analyzed statistically and are shown in Table 6. As can be seen, significant differences from control values were found in organ weights and organ to body weight ratios in the 5.0 mg/liter JP-4 rats. An increase in weight was found in lung, liver, spleen and kidney. Micropathological examination of these tissues failed to reveal any dose related effects which could be attributed to this increase in organ weights.

Two mice from the 5 mg/liter group had lung adenomas of the type commonly found in mice. One mouse from the high level group (not one of the two previously mentioned) was found to have lymphosarcoma in the lungs, spleen and lymph nodes with metastases to other organs. No tumors were found in any of the other species examined.

TABLE 6. EFFECT OF EXPOSURE TO 5 MG/LITER JP-4 VAPORS ON RAT ORGAN AND ORGAN/BODY WEIGHT RATIOS

Organ	Weight (g)	Ratio	Weight (g)	Ratio
Lung	2.23	0.466	2.38	0.493*
Liver	14.31	2.98	15.75*	3.27**
Spleen	0.90	0.187	1.02*	0.213*
Kidney	3.36	0.700	3.76*	0.782**

* Significant at 0.05 level.
** Significant at 0.01 level.

The only other significant pathological finding was an increase in the incidence of chronic murine bronchitis in the rats exposed to both concentrations of the jet fuel. Table 7 shows the incidence of this manifestation in the various exposure groups.

TABLE 7. INCIDENCE OF CHRONIC MURINE BRONCHITIS IN RATS EXPOSED TO JP-4 VAPORS

	Controls	Benzene Controls	5 mg/liter JP-4	2.5 mg/liter JP-4
Number Examined	24	25	30	29
Number with Bronchitis	0	2	8	8

After 11 months, approximately 70% of the rats held for post-exposure observation have died while only 37% of the mice have died. Mortalities were equally distributed between all exposure and control groups. Chronic respiratory disease was the principal cause of death. There were no gross lesions in any of these animals which could be attributed to exposure. The results of the histopathological examinations of these postexposure animals are not yet available. The surviving rats and mice are scheduled for sacrifice twelve months postexposure.

No significant number of deaths occurred during the 33 weeks of exposure. All body weights of the exposed groups were not statistically different from their respective control groups. Central nervous system effects were found in the benzene control and JP-4 exposed dogs during the early portion of the study. The dogs appeared to adjust to the effect and showed normal activity during the last 5 months of exposure. Mean organ weights as well as their ratios were significantly elevated in the high level jet fuel exposed rats when compared with their respective controls. Histopathology which included oil-red-o staining failed to reveal any fat deposition or abnormal alterations in any of the organ tissue which could account for the organ weight increases. Histopathologic findings in exposed monkeys, dogs and mice showed no treatment related effects. However, a suggestion of pulmonary irritation in the exposed rats is indicated by the incidence of chronic bronchitis.

An increase in osmotic erythrocyte fragility was seen in the female dogs exposed to 5 mg/liter jet fuel vapor for a period of 10 weeks, after which the effect diminished. The results of clinical hematology and chemistry tests performed on dogs and monkeys provide no evidence of kidney or liver toxicity from exposure to JP-4 jet fuel vapors.

Organ hyperplasia and bronchial irritation in rats and CNS effect and osmotic erythrocyte fragility increases in female dogs appear as the salient signs of toxic stress in this study. Although the reasons for the organ hyperplasia in rats is not clear, it appears to be of little toxicological significance as there was no tissue destruction or alteration. The increase in RBC osmotic fragility appears to have been a real effect of unknown etiology which was transient in nature. The central nervous system effect seen in dogs and respiratory irritation in rats should be considered relative to possible human experience of chronic exposure to JP-4 vapors. Either of these manifestations could result in a definite hazard to workmen either immediate, as in the case of the central nervous system effects, or long range in the case of the bronchial irritation which could lead to secondary bacterial infections.

Based on this data, it is suggested that workmen should not be allowed to inhale more than 2.5 mg/liter JP-4 vapors (12.5 ppm benzene equivalent) for extended periods of time, i.e., eight hours a day, 5 days

per week. It must be emphasized that this recommended standard is an estimate based on available experimental data and may be subject to modification by more extensive data collected from human experience.

A 6-Month Chronic Inhalation Toxicity Study of the Biological Effects of JP-9 Constituents

A new fuel has been developed for extending the flight range of aircraft before refueling. The fuel designated JP-9 is a mixture of three primary ingredients, namely, RJ-4, RJ-5, and methylcyclohexane. RJ-4 and RJ-5 are high density hydrocarbons yielding a greater BTU output per unit volume than conventional jet aircraft fuels. They also have a higher viscosity which causes pumping or flow problems at low temperatures which is the reason for the addition of methylcyclohexane to the mixture. The precise composition of the JP-9 fuel is not fixed but will be tailored for use in specific aircraft systems. Although no toxicity data are available for JP-9 fuel, it is not meaningful to evaluate the entire mixture for two reasons: first, the actual mixture has not been set, and second, methylcyclohexane is extremely volatile in comparison with the other constituents and would dominate the vapor exposure mixture, thus masking the effects of RJ-4 and RJ-5.

The acute and chronic toxicity studies on methylcyclohexane have been reported by Treon et al. (1943). Acute exposures of rabbits to inhaled concentrations of methylcyclohexane above 10,000 ppm (≈40 mg/liter) caused significant weight loss, narcosis and convulsions while a concentration of 15,227 ppm was fatal in slightly over one hour. Repeated daily, 5-hour exposures of rabbits to concentrations of 1162 ppm or lower for periods up to 10 weeks produced no measurable or observable signs of toxicity.

Some of the physical chemical properties of RJ-4 and RJ-5 are shown in Table 8. RJ-4 is a mixture of isomers of perhydromethylcyclopentadiene. RJ-5, also known as "Shelldyne H," is a mixture of reduced dimers of bicycloheptadiene. To examine the acute inhalation hazard, groups of six rats each were exposed for 6 hours to essentially saturated vapors (MacEwen and Vernot, 1974) of each compound. No adverse effects were seen during exposure. Pathologic examination after 14-day postexposure observation showed no abnormalities. Peroral doses of 4 g/kg RJ-5 in corn oil were not lethal to a group of 3 rats; however, 2 of 3 mice succumbed to a 250 mg/kg dose.

The toxicity of RJ-4 and RJ-5 has not been reported previously and it was, therefore, necessary to conduct chronic inhalation studies with these materials to evaluate their potential health hazard.

TABLE 8. PHYSICAL CHEMICAL PROPERTIES OF RJ-4 AND RJ-5

	RJ-4	RJ-5
Empirical Formula	$C_{12}H_{20}$	$C_{14}H_{20}$
Molecular Weight	164	188
Boiling Point (°F)	431	522
Vapor Pressure (70 F)	0.354 mm Hg	0.025 mm Hg
Density (70 F)	0.925 g/ml	1.0813 g/ml

Accordingly then, concentrations of 0.15 mg/liter RJ-5 and 2 mg/liter RJ-4 were selected for the 6-month chronic exposure of 4 animal species. The levels chosen are slightly below saturation vapor pressures so that condensation on chamber surfaces would not occur.

Each experimental group and the unexposed chamber controls consisted initially of 4 female and 4 male beagle dogs, 40 male CFE rats, 40 female CF-1 mice, and male and female Macaca mulatta monkeys, 4 per chamber.

Groups of animals were exposed in Thomas Domes operated with nominal airflows of 40 cfm at a slightly reduced pressure, 725 mm Hg, to avoid leakage of the hydrocarbons. Temperatures were controlled at 72 $\pm$ 2 F and relative humidity at 50 $\pm$ 10%. Exposures

were conducted on a 6 hour/day, 5 day/week schedule. No exposures were made on weekends and holidays. Upon completion of the daily exposures, the chambers containing RJ-4 and RJ-5 were purged with air for 30 minutes before lifting the dome tops. Cleaning of the chambers was done and residual food replaced with fresh supplies at this time.

Although expected to be low, the toxicities of the chemicals under study are unknown except for the minimal acute animal information mentioned earlier. Personnel working with these materials avoided skin contact and inhalation. The vapor generation apparatus and chemical supplies were in ventilated hoods and the areas were no smoking zones.

The chamber concentrations of RJ-4 and RJ-5 were continuously monitored using flame ionization hydrocarbon analyzers. The generation and monitoring techniques were identical to those used during the JP-4 toxicity study (MacEwen and Vernot, 1974).

To measure the chronic toxicity of RJ-4 and RJ-5, a limited number of parameters were selected, with the view toward increasing the variety of tests should the basic battery reveal trends or deleterious effects during the course of the study.

All exposed animals were observed for signs of toxic stress as well as mortality. Gross and histopathologic examination was made on all dead animals. Body weights of dogs, monkeys and rats were measured on a biweekly schedule. Table 9 shows the battery of clinical hematology and chemistry tests performed on blood samples taken from dogs and monkeys on a biweekly basis. A complete battery of clinical laboratory tests were made at the start and at the completion of the exposures. These tests include, in addition to those shown in Table 9: creatinine, chlorides, cholesterol, BUN, total inorganic phosphorus, bilirubin and serum triglycerides. At final blood sampling or sacrifice of the large animals, additional blood samples were drawn for identification and refrigerated storage of serum. These "banked" serum samples were stored until histopathology reports were received and reviewed. Twenty rats and mice from each of the study groups are being retained for 1-year observation following exposure termination in the event there are any postexposure effects from RJ-4 and/or RJ-5 inhalation. All remaining animals were sacrificed at exposure termination and submitted for gross histopathologic examination. Major organs were taken from 20 rats from each group and weighed to allow for comparison of mean organ weights and organ to body weight ratios.

TABLE 9. CLINICAL BLOOD TESTS PERFORMED ON RJ-4, RJ-5 EXPOSED AND CONTROL DOGS AND MONKEYS

HCT	Potassium	Alkaline Phosphatase
HGB	Albumin/Globulin	SGPT
RBC	Total Protein	Differential Cell Count
WBC	Calcium	
Sodium	Glucose	

A curious effect occurred in the rats exposed to RJ-4. Diarrhea was evident in the majority of the rats at 10 weeks of exposure and continued throughout the duration of the exposure portion of the study. Frequent postexposure observation of surviving rats revealed gradual alleviation of this condition. At 14 weeks postexposure, there were no signs of diarrhea.

There were 6 deaths during the 6 months of exposure. One male monkey died during the 7th week of exposure to RJ-5. Pathologic examination revealed death was due to gastric dilatation of unknown etiology, but believed to be unrelated to exposure. One mouse in each of the exposure groups was sacrificed because of accidental injuries which occurred during exposure. Remaining mortality was limited to control rodents. One mouse and one rat died of pneumonia at 1 and 24 weeks respectively. One rat was sacrificed at 10 weeks because of abnormal behavior indicative of middle ear infection.

Mean body weights of exposed monkeys measured on a biweekly schedule were normal when compared with control weights taken on the same time schedule. However, weight gain depressions were noted for rats and dogs exposed to RJ-4 and RJ-5.

The growth rate of rats is shown in Figure 1. Noticeable is the apparently subnormal gain from the second week of exposure forward for both exposed groups. The mean weights of the RJ-4 exposed rats were statistically different from control values at all time periods. Although at several time periods the mean weights of the RJ-5 exposed rats were 10-12 grams less than control, statistical calculations revealed no significant differences from control weights. The odors of RJ-4 and RJ-5 were very noticeable and objectionable even after purging of the chambers after each exposure period. It was theorized that the unpleasant odor of these compounds might cause appetite suppression in rats resulting in weight gain suppression. To test this possibility, food consumption measurements were made over a 3-day period during the 10th week of exposure. The daily results were variable, but overall, there was no real difference in food consumption between control and exposed rats.

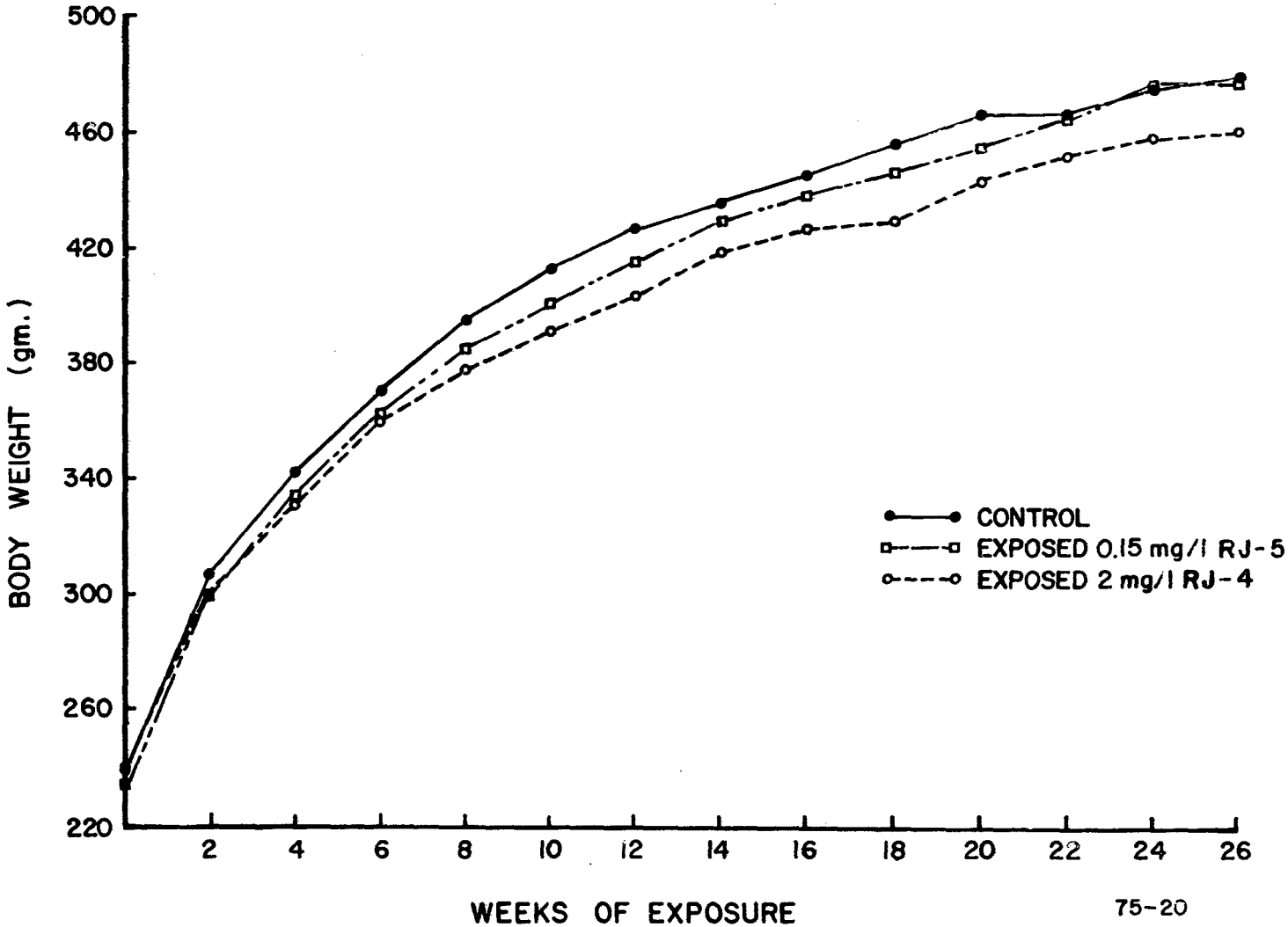


Figure 1. The effect of chronic inhalation exposure to RJ-4 and RJ-5 on rat growth rates.

Dog mean body weights are shown in Figure 2. Both exposed groups of dogs gained less weight than controls throughout the course of the study. At first glance, it would appear that weight gain supression was greater for the RJ-5 exposed dogs. However, the RJ-5 group weighed 0.5 kilograms less than the RJ-4 group at the beginning of the study. An examination of initial and final group mean body weights revealed that the controls, the RJ-5 and the RJ-4 groups gained 2.10, 1.22 and 0.98 kilograms respectively. Therefore, comparable subnormal weight gains occurred for dogs exposed to RJ-4 and RJ-5.

Biweekly clinical blood test results collected on dogs and monkeys showed no abnormalities or trends to adverse hematological effect.

There was no change in organ weights in rats exposed to RJ-5. Mean organ weights and organ to body weight ratios are shown in Table 10 for rats exposed to RJ-4 and unexposed controls. No toxicological significance is attached to the lung weight difference between RJ-4 exposed and control rodents since the body weights of RJ-4 exposed rats were also significantly lower than controls. However, mean liver and kidney weights as well as the ratios for the RJ-4 exposed rats are statistically higher than control values.

Figure 2. The effect of chronic inhalation exposure to RJ-4 and RJ-5 on dog growth rates.

TABLE 10. THE EFFECT OF 6-MONTHS CHRONIC INHALATION EXPOSURE TO RJ-4 ON RAT ORGAN[1] WEIGHTS

	Mean Organ Weight, Grams		Organ/Body Weight Ratio	
Organ	RJ-4	Control	RJ-4	Control
Liver	15.61*	12.96	3.536*	2.820
Kidney	3.50*	3.20	0.792*	0.674
Lung	1.92*	2.16	0.434*	0.471
Spleen	0.84	0.91	0.188	0.198
Heart	1.47	1.55	0.333	0.339

[1]Number = 20

*Statistically different from control at 0.01 level.

Gross pathology results for animals necropsied at exposure conclusion revealed no changes attributed to RJ-4 or RJ-5 exposure. There were no significant histopathology findings in monkeys and mice. However, acute inflammation was noted in the lungs of dogs and rats exposed to RJ-4 and RJ-5. This information is shown in Table 11. Lung lesions were restricted to male RJ-4 and female RJ-5 exposed dogs while 8 of 20 RJ-4 exposed rats and 6 of 20 RJ-5 exposed rats showed bronchopneumonia only.

TABLE 11. LUNG HISTOPATHOLOGY IN DOGS AND RATS EXPOSED TO JP-9 CONSTITUENTS (RJ-4 AND RJ-5)

	Condition		
	Bronchopneumonia	Bronchitis	Abscess
RJ-4 Exposed			
Dogs ♂	3/4	1/4	0/4
Dogs ♀	0/4	0/4	0/4
Rats ♀	8/20	1/20	0/20
RJ-5 Exposed			
Dogs ♂	0/4	0/4	1/4
Dogs ♀	2/4	3/4	0/4
Rats ♂	6/20	0/20	0/20
Controls			
Dogs ♂	0/4	0/4	0/4
Dogs ♀	0/4	0/4	0/4
Rats ♀	2/20	1/20	0/20

At 8 months postexposure, approximately 75% of the rats held for observation are dead. Mortality is evenly distributed among the control and both exposed groups. Mortality for mice is 2, 3 and 4 for controls, RJ-4 exposed and RJ-5 exposed respectively. Almost without exception, death in all groups of rats and mice was due to pneumonia.

The 6-month chronic inhalation exposure of four animal species to near vapor saturation concentrations of RJ-4 and RJ-5 was not fatal. Dogs and rats in both exposure groups experienced growth rate depression relative to their controls which was statistically significant except in the case of rats exposed to RJ-5 vapor. Mean liver and kidney weights as well as their ratios were significantly elevated in RJ-4 exposed rats when compared with control data. Histopathology which included oil-red-o staining failed to reveal any fat deposition or abnormal alterations in liver and kidney tissue which could account for increased organ weights in RJ-4 rats. Histopathologic findings in exposed monkeys and mice showed no abnormalities that were treatment related. However, for dogs and rats, considering acute pulmonary inflammation as a group, i.e., abscess, bronchopneumonia and bronchitis, the frequencies suggest respiratory irritation with the probability of secondary bacterial inflammation. The results of clinical hematology and chemistry tests performed on dogs and monkeys provide evidence that no septicemic injury occurred from chronic exposure to RJ-4 or RJ-5 vapors.

The results of this study demonstrate the low order of toxicity of JP-9 constituents exhibited in experimental animals. Kidney and liver hyperplasia in RJ-4 exposed rats and pulmonary irritation in dogs and monkeys exposed to RJ-4 and RJ-5 emerge as the salient results of this study. Although the reasons for organ hyperplasia in rats is not clear, it is of little toxicological significance in that there was no tissue destruction or alteration. The finding of respiratory irritation should be considered relative to possible human experience of chronic exposure to RJ-4 or RJ-5. In this regard, certain factors must be considered. Due to their low vapor pressures, the inhalation hazard (the probability of injury in use) is extremely low. The odors of RJ-4 and RJ-5 are extremely objectionable and it is, therefore, doubtful that workers would tolerate concentrations anywhere near those used in this study for any substantial time period. Furthermore, as constituents of JP-9 fuel, the toxicity of the mixture would be largely that of methylcyclohexane. However, as separate entities, both fuels show a low order of toxicity in experimental animals and are judged to be of a low inhalation hazard to man.

Acute Inhalation Toxicity of Hydrazine, Monomethylhydrazine and Unsymmetrical Dimethylhydrazine in Golden Syrian Hamsters

Studies to determine the carcinogenic potential of the chronic inhalation of hydrazine, monomethylhydrazine (MMH) and unsymmetrical dimethylhydrazine (UDMH) were planned by THRU to be conducted over the next two years. Animals to be used in these studies include rats, mice, hamsters, and dogs. The literature contains a substantial amount of inhalation toxicity data concerning hydrazine and its methylated derivatives (Rinehart et al., 1960; Comstock et al., 1954) on rats, mice and dogs but only one publication included acute toxicity data on hamsters. Jacobson et al. (1955) conducted four-hour inhalation exposures of hamsters to the vapors of MMH and UDMH. He reports the four-hour LC_{50}'s for the respective compounds as 143 and 392 ppm for hamsters. Jacobson also stated that hemolytic effects were found in dogs exposed to nonlethal concentrations of MMH.

This paucity of acute inhalation toxicity data on hamsters indicated the need for additional information including the determination of LC_{50} values for all three compounds. The experiments reported herein were conducted to supplement the existing inhalation toxicity data and to serve as a basis for the establishment of concentrations to be used in the future chronic exposure studies.

The reactive nature of these compounds required an introductory system specifically designed to minimize chemical breakdown and ensure that the hamsters were being exposed to the unreacted material. The chemical introduction system is shown in Figure 3. The entire chamber air flow (1 to 2 cfm) is sent through the evaporator which consists of a heated teflon line. The line is heated to a temperature which eliminates pooling of the liquid but does not cause charring.

Figure 4 is a schematic of the analytical system used for all three compounds. The coil is positioned in the chamber during analysis for quick response and to minimize loss along the sample lines. A 2.5 mM I_2/L solution was used and the colorimeter had 520 nm filters. The analytical system was calibrated daily using standards prepared in Mylar® and/or Teflon® bags filled with measured amounts of dry nitrogen into which the desired amount of compound was injected through a rubber septum. Gentle warming and manipulation of the bag ensured complete evaporation and mixing.

Groups of ten male, golden syrian hamsters were exposed to various concentration levels of the contaminants for one-hour periods. All exposures were conducted in a specially constructed 3.5 ft.^{3} Plexiglas®

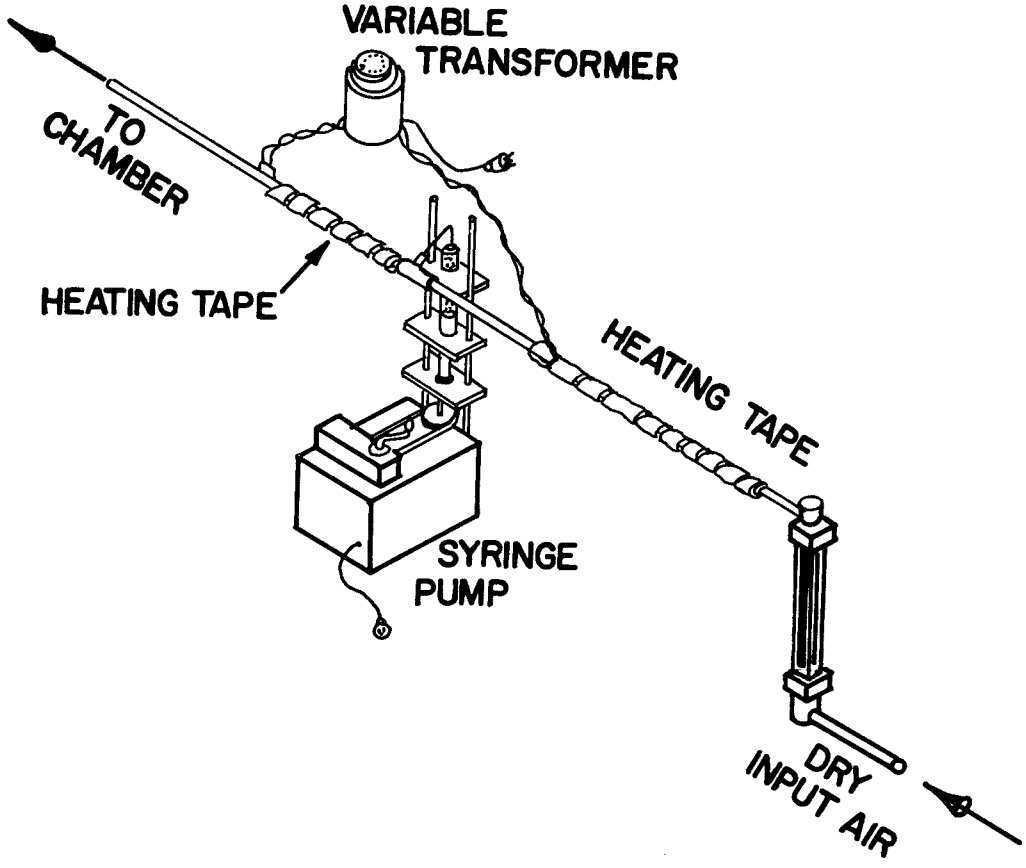


Figure 3. Schematic diagram of contaminant introduction system for acute toxicity evaluation of the hydrazines.

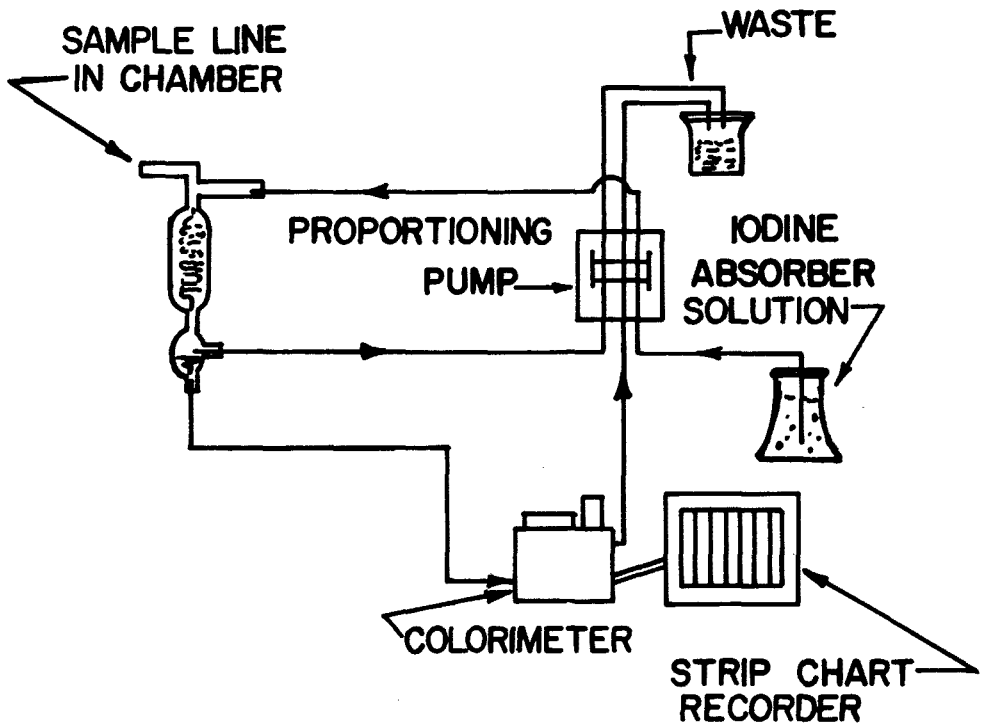


Figure 4. Schematic diagram of the analytical system used for continuous monitoring of the hydrazines during acute toxicity studies.

chamber. This chamber was fitted with a sliding cage drawer to allow for rapid insertion and withdrawal of the test animals. The desired concentration of the contaminant was first obtained within the chamber, and the hamsters to be exposed were then introduced into the chamber by means of the sliding cage drawer.

The hamsters were examined during and after exposure for visible signs of toxic stress and mortality. Hamsters that survived the exposure period were held for 14 days. Gross and histopathological examinations were made on representative numbers of hamsters which either died as a result of exposure or were sacrificed following the 14-day observation period. Deaths which occurred during the 14-day observation period were included in the final mortality ratio which was used for calculation of the LC_{50} value. The LC_{50}'s were calculated using the probit analysis method of Finney (1952).

Following establishment of each LC_{50}, a group of five hamsters was exposed to the lowest concentration used for calculation of the respective LC_{50}'s for standard hematological examination. Blood samples were taken from each of these hamsters via heart puncture immediately following exposure. A control group of five hamsters was examined simultaneously.

Hamsters exposed to all three compounds showed immediate eye and nose irritation followed by labored breathing and gasping. Coordination was noticeably affected but only in the hydrazine exposures did the animals become prostrate. Loss of consciousness did not occur in any of the exposures. Except for prostration in the high level hydrazine exposures, all of the above signs of toxic stress were seen in all exposures; however, time of effect was concentration related with the signs being manifested earlier in the highest level exposures.

Convulsions occurred during the last few minutes of the highest level MMH exposures and continued for as long as one hour postexposure. No convulsions occurred during exposure to UDMH; however, convulsions were seen postexposure, usually preceded by rapid running about the cage. Convulsions did not occur in hamsters exposed to hydrazine vapor, either during or after exposure.

Alopecia was a common finding in the hamsters following exposure to hydrazine vapors. This was seen in all survivors regardless of the concentration received. The hair loss was confined primarily to the back and facial areas. The abdomen and legs were unaffected.

Deaths following exposure to lethal concentrations of UDMH and MMH occurred within 24 hours. If they survived this critical period, the hamsters continued to live for the remainder of the 14-day postexposure observation period. This was not the case with hamsters exposed to lethal concentrations of hydrazine vapors. Many of these deaths were delayed, some as long as 11 days postexposure (Tables 12-14).

All hamsters surviving near lethal concentrations of these compounds showed severe weight losses. Even those exposed to nonlethal concentrations showed a depressed growth rate. Hematology results from hamsters exposed to the lowest concentrations of the compounds showed no significant changes in hematocrit, hemoglobin, red blood cell count, white blood cell count or variable neutrophils and lymphocytes when compared to the control hamsters.

Histopathological examination of the tissues from the animals exposed to UDMH vapor showed severe congestion of the lungs with dystrophy of the bronchial epithelium. This effect increased in severity with increased exposure concentrations. The livers were congested (moderate to severe) and the kidneys showed degeneration of the distal convoluted tubule epithelium. Necrosis of the Henles loop tubule epithelium at the cortico-medullary junction was also a common finding in

TABLE 12. SUMMARY OF TOXIC EFFECTS FROM ONE-HOUR MMH INHALATICN EXPOSURES TO HAMSTERS

Conc., ppm	Mortality Ratio	Time to Death	14-Day Body Weight Effect
1380	9/10	6 at 3 hrs., 3 at 10 hrs.	Survivor lost 32 grams
1110	7/10	3 at 1 hr., 4 at 17 hrs.	1 - loss of 15 grams 2 - suppressed weight gain
910	2/10	1 at 2.5 hrs., 1 at 18 hrs.	Mean loss of 6 g/animal
810	2/10	2 at 18 hrs.	Mean loss of 7 g/animal
620	2/10	2 at 18 hrs.	Mean loss of 4 g/animal
460	0/10	-	Suppressed weight gains

LC_{50} and 95% Confidence Limits = 991 (870-1130) ppm

or 1.86 (1.64-2.13) mg/L.

TABLE 13. SUMMARY OF TOXIC EFFECTS FROM ONE-HOUR UDMH INHALATION EXPOSURES TO HAMSTERS

Conc., ppm	Mortality Ratio	Time to Death	14-Day Body Weight Effects
3300	10/10	All within 3.5 hrs.	--
2800	5/10	4 at 4 hrs., 1 at 10 hrs.	Suppressed weight gains
2540	8/10	All within 24 hrs.	Suppressed weight gains
2230	4/10	All within 10 hrs.	Mean gain 5 g/animal
1920	5/10	All within 24 hrs.	Mean gain 5 g/animal
1600	0/10	--	Suppressed weight gains

LC_{50} and 95% Confidence Limits = 2271 (2055-2509) ppm

or 5.80 (5.25-6.41) mg/L.

TABLE 14. SUMMARY OF TOXIC EFFECTS FROM ONE-HOUR HYDRAZINE INHALATION EXPOSURES TO HAMSTERS

Conc., ppm	Mortality Ratio	Time to Death	14-Day Body Weight Effects
2770	9/10	3 within 1 hr., 4 within 15 hrs., 1 at 3 days, 1 at 4 days	Survivor lost 19 grams
2450	3/10	1 at 12 hrs., 1 at 1 day, 1 at 3 days	Mean loss of 31 g/animal
2140	3/10	1 at 5 min., 1 at 1 day, 1 at 2 days	Mean loss of 18 g/animal
1920	3/10	1 at 1 day, 1 at 3 days, 1 at 11 days	Mean loss of 32 g/animal
1600	2/10	1 at 10 hrs., 1 at 11 days	Mean loss of 16 g/animal
1280	2/10	1 at 8 days, 1 at 12 days	Mean loss of 21 g/animal

LC_{50} and 95% Confidence Limits = 2585 (1935-2941) ppm
or 3.38 (2.53-3.85) mg/L.

these animals. Although a dose-response effect was seen in the pulmonary pathology, liver and kidney damage appeared to be just as severe at the lowest concentration to which hamsters were exposed as at the highest concentration.

Gross examination of the hamsters exposed to MMH vapors showed both lung and liver congestion. Histological examination revealed pathologic findings largely limited to kidney, liver and lungs. The degree of alveolar wall irritation ranging from congestion to edema to hemorrhage reflects a dose dependent response to MMH vapor. Lung congestion and edema was seen more frequently at the high dose levels while lung hemorrhage occurred only after exposure to the two highest levels. Hamsters exposed to the higher dose levels showed more severe changes in the epithelium of the tracheobronchial tree, i.e., cuboidal rather than columnar epithelial atrophy plus the appearance of erosion and ulcerations. Catarrhal inflammation, indicative of mild irritation, was largely confined to the lower dose levels.

Kidney and hepatic congestion was noted in animals from all exposure levels but the degree of severity and incidence of the lesion does not appear to be dose related.

Gross examination of the hamsters exposed to hydrazine (N_2H_2) vapors revealed moderately congested lungs and congested, friable livers, mostly at the higher exposure levels. Blood tinged urine and a yellow discoloration of the liver was also seen in the hamsters exposed at the highest concentrations. Histological examination of the tissues revealed pathologic findings largely limited to the kidney, spleen, and liver.

Evidence of respiratory irritation was seen in most dose levels as either congestion, tracheobronchial epithelial dysplasia or atrophy and/or evidence of alveolar insult seen as thickening of alveolar walls.

Kidney congestion was seen commonly in the low N_2H_4 dose groups. Changes indicative of intravascular hemolysis were seen, i.e., blood tinged urine and presence of red staining material (presumed to be hemoglobin) in Bowman's space and within kidney tubules. Further evidence of hemolysis was seen in the red pulp of the spleen in the presence of fine smooth particulate, red-staining material.

Liver congestion was noted especially in the low dose groups. Fatty degeneration was seen in the high dose groups being most severe in the 2450 and 2770 ppm exposed animals.

Of the three compounds tested, monomethylhydrazine was more toxic to hamsters than either hydrazine or unsymmetrical dimethylhydrazine. All three compounds caused severe respiratory irritation and central nervous system effects. Convulsions were produced after exposure to MMH and UDMH but not following exposure of hydrazine vapors. However, exposure to hydrazine vapors, regardless of concentration, resulted in severe hair loss to hamsters.

All three compounds cause significant lung, liver and kidney damage, even at the lowest levels tested in this study. Although no hematological effects were measured in the lowest dose groups examined immediately after exposure, hemosiderin deposits were found in the livers of hamsters exposed to high level hydrazine vapors. The results of these tests indicate that although the hamster is less susceptible than rats or mice, they react to toxic levels of these three compounds in a manner similar to that reported for other species.

Continuous Animal Exposure to a Mixture of Dichloromethane and 1, 1, 1-Trichloroethane

An investigation of the effects of combined exposure of animals to dichloromethane and 1, 1, 1-trichloroethane was conducted using atmospheric concentrations of each solvent which had individually produced minimal measureable effects on livers. This study was undertaken at the request of the National Aeronautic and Space Administration to determine if previously established spacecraft TLV's (threshold limit values) for the individual solvent compounds were valid when both were present in an astronaut's breathing environment under continuous exposure conditions.

Exposure concentrations selected for this study were based on the results of 100-day continuous exposures to various animal species as reported by MacEwen et al., 1972, Haun et al., 1972, Weinstein et al., 1972 and MacEwen and Vernot, 1973 in which concentrations of 100 ppm dichloromethane and 1000 ppm 1, 1, 1-trichloroethane independently produced comparable degrees of liver triglyceride levels and fat accumulation in livers of mice.

Two groups of animals consisting of 4 Rhesus monkeys, 8 beagle dogs, 40 Sprague-Dawley CFE rats and 180 ICR - CF-1 mice were housed in Thomas Dome exposure chambers where one group served as controls for the experimental group.

All dogs, monkeys and rats were weighed on a biweekly schedule at which time blood samples were drawn from the dogs and monkeys for hematology and clinical chemistry determinations listed below:

HCT	Sodium	Glucose
HGB	Potassium	Alkaline Phosphatase
RBC	Calcium	SGPT
WBC	Albumin	SGOT
Blood Indices	Total Protein	

Subgroups of 10 mice each were serially sacrificed on a weekly schedule from both control and exposed groups for gross pathologic examination, body weight, liver weight, and liver triglyceride determinations. Histologic preparations were also examined for liver fat content. These samples were taken throughout the exposure period of 13 weeks and for 2 additional postexposure weeks to determine reversibility of any observed effects.

At the conclusion of the exposure period all rats and monkeys were necropsied for complete histopathologic examination as were most of the dogs. Two control and 2 exposed dogs were held one month post-exposure, again for the purpose of determining reversibility of hematologic or clinical chemistry changes.

During the 90-day experimental period the animals were fed ad libitum at daily intervals with the remaining food discarded and replaced to minimize solvent adsorption and subsequent oral intake by the animals.

The solvents used in animal exposures were technical grade obtained in 55 gallon drums. Both the dichloromethane and the 1,1,1-trichloroethane were pumped from the drums into a heated air duct and the resulting vaporized mixture was then introduced into the chamber air supply duct. The chamber concentrations were continuously monitored to permit control by adjusting the speed of the liquid transfer pumps.

Although chamber concentrations of each solvent had been satisfactorily analyzed with a flame ionization hydrocarbon analyzer in the independent studies, the hydrocarbon analyzer could not be used for this comparative study since it could not differentiate between the 2 compounds. Since the solvents are relatively unreactive and had good

infrared spectra, 2 single beam IR analyzers with variable wavelength and pathlengths (Miran I®) were obtained for this purpose. Noninterfering absorbance bands were selected for each compound from full range infrared scans. Air samples were drawn from either the control or exposure chamber through a 3-way valve and passed through the 2 IR analyzers in series determining the concentration of each solvent in sequence.

The solvent introduction and analysis systems are shown in Figure 5. The air sampled from the control animal chamber was utilized to set the zero baseline for the analyzers. The instruments were calibrated daily against precisely measured standards mixed in gas bags which spanned the expected range of each solvent. The specific instrument conditions used are listed in Table 15. The nominal air concentration selected for each solvent was 100 ppm of dichloromethane and 1000 ppm of 1,1,1-trichloroethane. The actual mean concentration for the 90-day exposure period was 99.6 ppm dichloromethane and 993 ppm 1,1,1-trichloromethane. No fluctuations of solvent concentrations in the exposure chamber exceeded 10% of the preselected level during the course of the study.

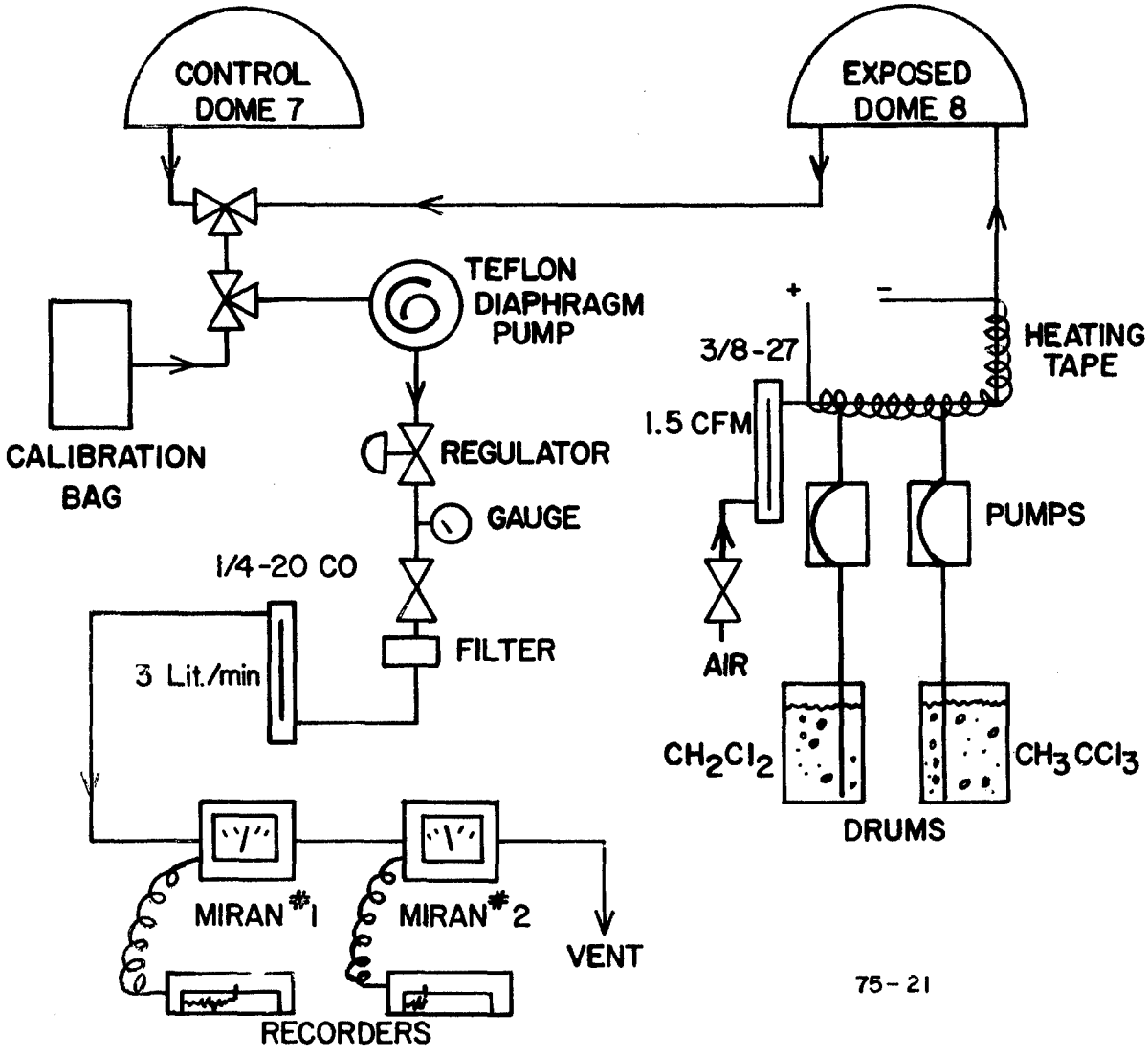


Figure 5. Mixed solvent inhalation chamber contaminant introduction and monitoring systems.

TABLE 15. STANDARD INSTRUMENTAL SETTINGS FOR CONTINUOUS IR MONITORING OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE

Control Control	IR Analyzer-I (Dichloromethane)	IR Analyzer-II (1,1,1-Trichloroethane)
Slit Width	0.50 mm	0.50 mm
Wavelength	7.78μ	13.8μ
Pathlength	6.75 m	0.5 m
Absorbance	0.1 A	1 A
Gain	10 X	10 X
Time Constant	1	10
Cut Off Frequency	-	0.05

The growth rate of rats was not significantly affected by exposure to the solvent mixture as shown in Figure 6, and in mice a significant difference between control and exposed group weight was only seen at the sixth week of the experimental period (Table 21). The difference between test and control groups, although not statistically significant, was also greatest at the six week point for the rats as well as for dogs and monkeys as shown in Table 16.

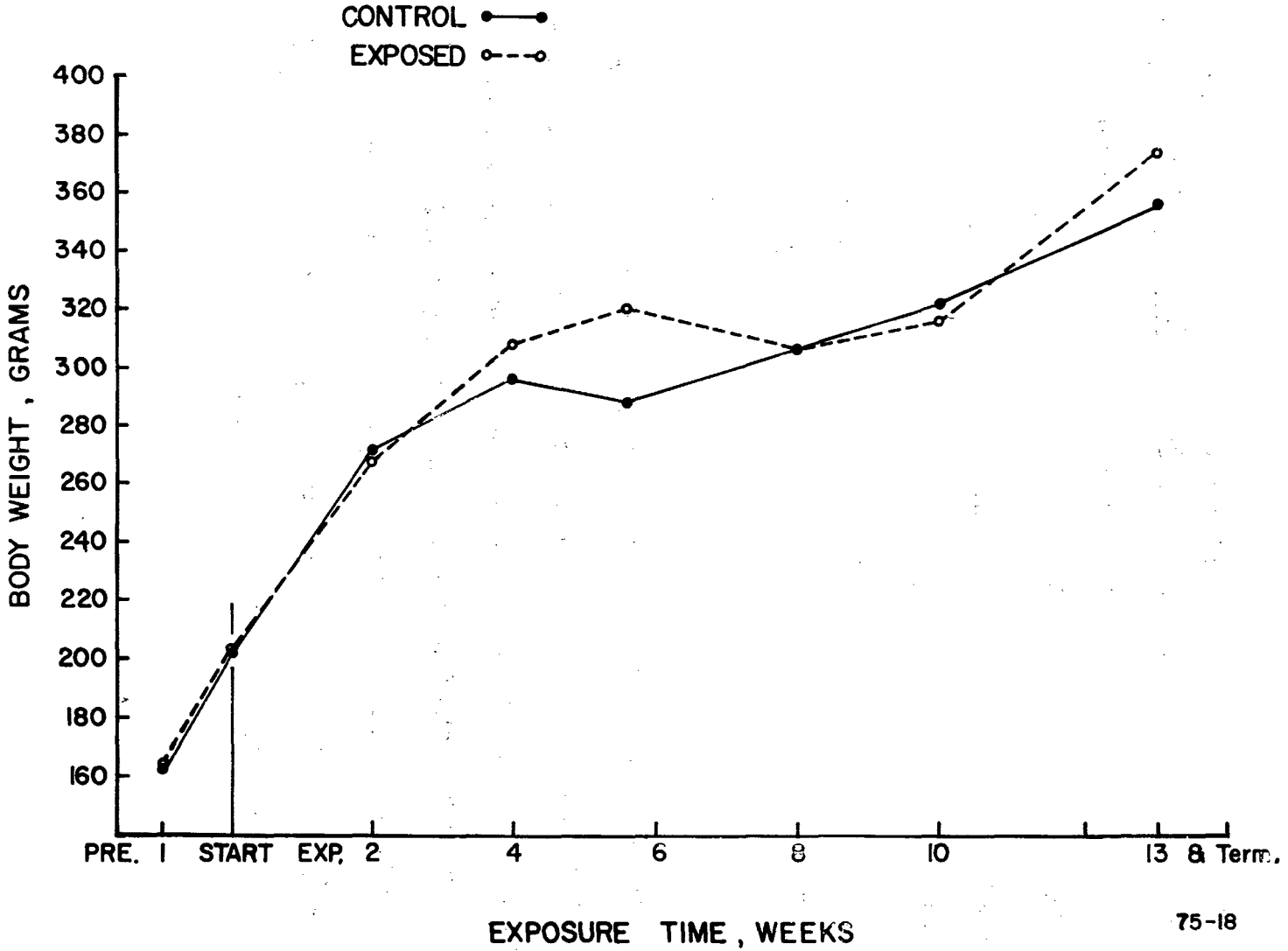


Figure 6. Effect of continuous exposure to dichloromethane and 1, 1, 1-trichloroethane on rat growth.

TABLE 16. EFFECT OF CONTINUOUS MIXED SOLVENT INHALATION EXPOSURE ON DOG AND MONKEY BODY WEIGHTS

(Mean Body Weight in Kilograms)

Exposure Length Weeks	Dogs (N = 8)		Monkeys (N = 4)	
	Control	Exposed	Control	Exposed
-2	8.90	8.64	3.51	3.42
0	9.09	8.68	3.30	3.30
2	10.05	9.45	3.53	3.35
4	10.36	9.57	3.62	3.67
6	10.69	9.60	3.78	3.49
8	10.89	9.72	3.81	3.61
10	10.63	9.78	3.82	3.75
13	10.77	10.18	3.65	3.49

Clinical chemistry values for monkeys (Table 17) show no significant differences between test animals and their air exposed controls. Dogs and monkeys, however, showed slightly increased hematocrit, red cell counts and hemoglobin values during exposure to the mixed solvents (Tables 18 through 20). These changes were accompanied by a slight rise in reticulocyte counts and a slight decrease in mean corpuscular hemoglobin (MCH). These findings are consistent with the finding of increased carboxyhemoglobin levels in exposed dogs and monkeys. The mean carboxyhemoglobin value in dogs was 0.9% saturation and in monkeys was 1.2%. These results are slightly lower than that reported for dichloromethane alone (MacEwen et al., 1972) and may represent a lower metabolic conversion of dichloromethane to carbon monoxide in the presence of 1,1,1-trichloroethane.

Dogs exposed to the mixture of solvents had a significant increase in serum alkaline phosphatase and a decrease in serum glutamic oxaloacetic transaminase (SGOT) both of which returned to control levels during the postexposure observation. The biological significance of these changes is difficult to access since the decreased SGOT values were still within the normal range and although the increase in alkaline phosphatase may indicate a mild hepatotoxic response, there were no other changes observed in dogs to support such a conclusion.

TABLE 17. MEAN CLINICAL CHEMISTRY VALUES FOR MONKEYS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	Sodium (MEq/L)		Potassium (MEq/L)		Calcium (mg %)		Albumin (g %)		Total Protein (g %)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure										
-4	153	156	3.9	4.3	9.9	16.1	4.7	4.6	7.9	7.9
-0	150	152	4.1	4.6	10.0	10.1	4.6	4.8	7.8	8.1
Exposure										
2	149	151	4.9	5.9	10.5	10.6	4.4	4.2	7.7	7.8
4	156	154	4.8	5.1	11.4	11.1	4.7	4.8	8.1	8.3
6	156	157	5.1	5.2	11.4	11.3	4.3	4.4	7.6	8.0
8	155	158	5.2	5.3	11.4	11.3	4.4	4.2	7.8	7.7
10	154	155	4.6	5.4	11.2	11.7	4.5	4.7	7.9	8.1
13	158	156	5.0	5.5	11.5	11.3	4.9	5.0	8.6	8.4

TABLE 17. CONTINUED

Weeks	Glucose (mg %)		Alk. Phos. (KA)		SGPT (RF)		SGOT (Int'l Units)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	79	91	37	60	35	37	33	36
-0	87	74	46	58	36	41	34	45
Exposure								
2	101	93	28	41	32	38	30	30
4	112	101	30	41	35	37	37	35
6	101	121	29	42	37	36	28	37
8	93	116	30	36	33	30	24	34
10	99	105	30	37	32	33	32	34
13	94	114	37	36	32	48	33	56

TABLE 18. MEAN HEMATOLOGY VALUES FOR MONKEYS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	HCT (vol %)		RBC (millions)		HGB (g %)		WBC (thousands)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	40	41	5.5	5.5	13.2	13.7	7.6	11.3
-0	39	40	5.6	5.6	13.2	13.6	8.7	13.0
Exposure								
2	43	40	5.7	5.8	13.8	13.2	9.4	14.9
4	41	40	5.4	5.6	13.1	13.2	12.9	12.1
6	43	40	5.7	5.6	13.7	13.3	11.5	13.2
8	42	38	5.8	5.5	13.7	12.7	11.0	14.8
10	42	40	5.6	5.6	13.6	13.2	7.9	12.4
13	42	40	5.8	5.8	13.4	13.7	10.1	9.2

TABLE 18. CONTINUED

Weeks	Reticulocyte (%)		MCV (cm)		MCH (uug)		MCHC (%)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	0.9	0.6	72.9	76.1	24.1	25.2	33.1	33.1
-0	0.6	1.0	71.8	72.2	24.2	24.5	33.7	34.0
Exposure								
2	0.6	0.7	75.1	69.4	24.3	22.8	32.4	32.8
4	1.2	0.9	76.1	71.3	24.6	23.4	32.3	32.9
6	0.8	1.3	74.4	71.7	23.9	24.0	32.1	33.5
8	1.2	0.6	72.5	69.6	23.6	23.0	32.6	33.1
10	0.7	1.1	74.1	71.9	24.3	23.7	32.8	33.1
13	1.1	1.8	71.8	68.1	23.2	22.8	32.3	33.4

TABLE 19. MEAN CLINICAL CHEMISTRY VALUES FOR DOGS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	Sodium (MEq/L)		Potassium (MEq/L)		Calcium (mg %)		Albumin (g %)		Total Protein (g %)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure										
-4	149	150	4.8	4.8	10.2	9.8	3.1	2.9	6.2	6.1
-0	147	147	4.5	4.7	9.8	9.5	3.2	3.0	6.3	6.1
Exposure										
2	148	150	5.0	4.9	10.0	9.4	2.8	2.5	6.3	6.0
4	151	150	5.1	5.3	10.0	9.9	3.1	3.0	6.6	6.3
6	154	152	5.2	5.0	11.0	10.5	3.0	2.8	6.2	6.1
8	150	152	4.9	5.0	10.6	10.4	3.0	2.7	6.4	6.2
10	150	148	4.9	5.0	10.8	10.2	3.2	3.0	6.7	6.4
13	152	151	5.1	4.8	11.0	10.3	3.2	3.2	6.9	6.6
Post-Exposure										
2	154	152	4.8	5.2	10.8	10.2	3.5	3.1	6.6	6.4
5	153	155	4.6	5.1	10.5	10.1	3.6	3.1	6.7	6.4

TABLE 19. CONTINUED

Weeks	Glucose (mg %) Test	Glucose (mg %) Control	Alk. Phos. (KA) Test	Alk. Phos. (KA) Control	SGPT (RF) Test	SGPT (RF) Control	SGOT (Int'l Units) Test	SGOT (Int'l Units) Control
Pre-Exposure								
-4	108	106	4.5	4.6	31	29	--	--
-0	105	103	4.6	4.4	33	31	38	41
Exposure								
2	82	94	5.9	4.7	33	37	20**	36
4	97	105	6.1**	4.2	32	38	16**	36
6	90	107	6.8**	4.4	28	36	16**	37
8	105	106	7.7**	4.8	24	28	13**	31
10	104	101	7.3**	4.5	27	31	16**	38
13	115	118	7.5**	3.9	27	32	15**	29
Post-Exposure								
2	110	89	5.8	3.8	28	31	21	28
5	121	109	4.0	3.9	20	25	25	31

TABLE 20. MEAN HEMATOLOGY VALUES FOR DOGS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	HCT (vol %)		RBC (millions)		HGB (g %)		WBC (thousands)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	44	44	6.1	6.1	14.6	15.0	11.0	11.5
-0	41	42	6.0	6.1	14.3	14.4	10.4	12.5
Exposure								
2	47	42	6.5	6.0	15.7	14.4	14.8	15.9
4	47	44	6.7	6.2	15.8	14.9	15.3	14.4
6	47	43	6.8	6.1	15.4	14.7	14.8	14.4
8	46	43	6.9	6.1	15.5	14.5	14.1	13.5
10	48	45	7.2	6.3	16.4	15.5	14.0	13.6
13	47	45	7.1	6.1	16.1	15.4	12.2	12.7
Post Exposure								
2	48	48	5.6	5.9	15.4	15.9	6.7	11.3
5	45	46	6.7	6.6	15.0	15.3	7.4	11.8

TABLE 20. CONTINUED

Weeks	Reticulocyte (%)		MCV (cm)		MCH (uug)		MCHC (%)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	0.7	0.6	72.5	72.7	25.0	24.6	33.2	33.9
-0	0.4	0.4	69.0	68.6	23.9	23.7	34.7	34.5
Exposure								
2	0.8	0.5	72.1	70.3	24.3	24.0	33.7	34.2
4	1.2*	0.7	70.0	71.2	23.7	24.1	33.8	33.8
6	0.9	0.8	69.4	69.9	22.8*	23.9	32.8	34.3
8	0.9	0.6	67.3	70.3	22.8*	23.8	33.8	33.9
10	0.9	0.6	67.1	70.9	22.9**	24.6	34.1	34.8
13	0.8	0.7	66.1	73.8	22.7**	25.3	34.4	34.3
Post-Exposure								
2	0.8	0.6	84.8	81.8	27.5	27.2	32.4	33.1
5	0.4	0.5	65.6	68.9	21.8	23.2	33.2	33.6

Sections of mouse liver from the sacrifice group were stained with Oil-red-O at weekly intervals during the course of the study. The livers had begun to accumulate some fat droplets in a diffuse pattern after one week of continuous exposure to the solvent mixture.

There was a slow but steady increase in liver fat content during the 13 weeks of exposure reaching a 2.5 + average on a scale of 0 to 4 + by the end of 90 days. Of ten mice held for one week postexposure only one showed any residual trace of fatty deposition and by the second post-exposure week the mouse livers were completely clear of fat with only a mild inflammatory response remaining.

The liver weights of the exposed mice were slightly heavier than those of the controls and the liver to body weight ratios (Table 21) of the exposed mice became significantly higher than those of controls after three weeks. The significance of the difference between exposed and control animals dropped from the 0.01 to 0.05 confidence level by the end of one week postexposure but was not reversed completely by the end of the second postexposure week. Although liver triglyceride values of the exposed mice appeared to be higher than control values, an extremely wide variability in analytical measurements made the results unsuitable for comparative purposes.

TABLE 21. EFFECT OF CONTINUOUS MIXED SOLVENT INHALATION EXPOSURE ON MOUSE GROWTH RATE AND LIVER WEIGHT

Weeks Exposed	Body Weight (grams)		Liver Weight (grams)		Liver/Body Weight	
	Test	Control	Test	Control	Test	Control
1	23.1	24.2	1.3	1.1	.049	.054
2	24.9	27.9	1.5	1.5	.058	.054
3	26.4	30.1	1.5	1.5	.057*	.050
4	27.6	30.4	1.5	1.5	.055*	.049
5	29.2	30.4	1.6	1.6	.054	.051
6	28.3*	31.2	1.5	1.5	.052	.047
7	30.4	31.6	1.8	1.5	.058**	.048
8	29.3	31.2	1.7	1.7	.058	.054
9	31.4	32.6	1.7	1.6	.054**	.047
10	30.2	35.0	2.0	1.7	.065**	.048
11	32.1	31.8	2.0**	1.4	.062**	.045
12	32.0	31.9	1.9*	1.6	.059**	.049
13	30.3	33.7	1.7	1.4	.046**	.042
Post-Exposure						
1	32.2	33.5	1.7	1.5	.053*	.045
2	31.3	34.5	1.6	1.4	.051*	.040

Rat organ weight measurements, shown in Table 22, taken at the conclusion of the 90-day exposure period exhibited an increase in liver; spleen and kidney weights and organ to body weight ratios of exposed animals of which the splenic gross weight and organ to body weight splenic ratio reached the 0.05 significance level.

Liver tissue specimens taken from mice killed at weekly intervals were prepared for electron microscopic evaluation of the progress of the lesion. These studies are incomplete at the present time and will be presented in a supplemental report.

At necropsy there were no apparent differences between the control and mixed solvent exposed dogs. This finding was confirmed by complete histologic examination of selected tissues from heart, lung, brain, liver, spleen, kidney, adrenals and testes.

In monkeys only one animal exposed to the solvent mixture showed a slight increase in the amount of fat deposited in hepatocytes of the periacinal region. In other monkeys the only lesion observed was that produced by lung mites in both exposed and control groups.

The common pathologic finding in both control and exposed rat groups was chronic respiratory disease (CRD) ranging from a mild to moderate state in those animals necropsied at the termination of the

TABLE 22. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE ON RAT ORGAN WEIGHTS

	Mean Organ Weight* (grams)		Mean Organ/Body Weight Ratio**	
	Exposed	Control	Exposed	Control
Heart	1.26	1.27	0.363	0.373
Lung	2.57	2.44	0.751	0.720
Liver	11.0	10.2	3.174	2.968
Spleen	0.80**	0.71	0.230**	0.205
Kidney	2.74	2.55	0.788	0.744

* N = 20

** Different from the control mean at the 0.05 significance level.

90-day study. One exposed rat that died during the 6th week of the study had increased fat deposition in the periacinal region rated at 3+ on a 0 to 4+ scale. Most exposed rats had scattered areas of renal tubular dilatation containing a pink staining amorphous material. This effect was seen in a few control rats. Oil-red-O stains of liver tissue were negative for fat.

The only significant finding in this study was the difference between control and exposed mice. There was a consistent finding in the liver tissue of exposed mice of multifocal periacinal areas in which there was vacuolization of surrounding hepatocytes. There were increased amounts of fat in these periacinal areas demonstrated with Oil-red-O stain. This effect was demonstrated to be reversible and cleared up within 14 days postexposure.

The combined effect of 90-day continuous exposure of animals to 100 ppm dichloromethane and 1000 ppm 1, 1, 1-trichloroethane does not appear to be greater than the effect of each alone. While the exposed mouse livers appeared to contain slightly more fat, the degree of increased liver weight and the liver to body weight ratios are slightly lower than those measured for each solvent alone. The metabolic conversion of dichloromethane to carbon monoxide with subsequent carboxyhemoglobin formation and accompanying hematologic changes is also slightly lower in the mixed exposures than was observed in studies with dichloromethane alone. Thus, the spacecraft TLV's for these two common industrial solvents appear to have been set at a safe level for either alone or the combination of both.

An Acute Toxicity Study of SYFO

A military compound designated "SYFO" has shown a high potential for use both as a rocket propellant ingredient and as an explosive material. Because no toxicity information existed on this compound acute toxicity data was desired to evaluate the potential hazard for personnel working with this material.

SYFO was received at this laboratory diluted in methylene chloride which reduced its shock sensitivity and made it safe for handling and shipment. A single dose oral LD_{50} study was initiated as the first step in assessing the toxic hazard of SYFO which was transferred to corn oil and the methylene chloride removed by gentle warming. This treatment resulted in a SYFO suspension in the corn oil which was kept suspended by continuous stirring on a magnetic stirring platform.

Glass syringes with special oral dosing needles were used to administer the SYFO to rats which were fasted for 16 hours prior to administration of the oral dose. This allowed for uniform absorption in all animals since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition. Five rats were dosed at each concentration level and the LD_{50} calculated using the moving interpolation method of Weil (1952).

Deaths which occurred during the 14 days immediately following the administration of the single oral dose were included in the final mortality Table 23). All that survived the 14-day postexposure observation period were sacrificed at that time.

Gross and histopathological examinations were done on any rat which died following the administration of the oral dose. Gross examination only was done on the rats that survived the 14-day postexposure observation period.

TABLE 23. MORTALITY RESPONSE OF ALBINO RATS TO SINGLE ORAL DOSES OF SYFO

Dose Level, mg/kg	Mortality Ratio*
2000	5/5
1000	2/5
500	0/5
250	0/5

LD_{50} and 95% confidence limits = 1072 (725-1585) mg/kg

*Number that died over the number dosed.

Gross examination of the rats which died following a peroral dose revealed no abnormal lesions. Microscopic examination revealed mild to moderate renal, hepatic and pulmonary congestion with scattered, mild intraalveolar hemorrhage.

Although the rat oral LD_{50} for SYFO (1072 mg/kg) would fall in the toxic category in most classification systems it is the upper end of the range and may be considered as only mildly toxic to the rat. Further studies were not undertaken with SYFO because it has extremely low volatility and it would be impossible to achieve a vapor concentration high enough to produce toxic effects at room temperature.

Percutaneous, Oral and Inhalation Studies for Toxicity Classification of Transportable Chemical Agents

Certain materials being transported do not have adequate toxicological data necessary for proper classification by the Department of Transportation. These materials were tested in this laboratory to verify the suitability of proposed transportation health hazards classification criteria. This was done by determining experimentally the 14-day oral LD_{50} in male and female rats, the 14-day toxicity by skin absorption, skin corrosion effects on rabbits, and when possible, the one-hour inhalation LC_{50} to male and female rats.

The toxicity classification system published in a previous report by Back et al. 1972, was used to categorize the compounds in the present study. The following criteria were used to determine the category into which each compound was placed.

	Extremely Toxic	Highly Toxic	Toxic
Inhalation, 1-hour LC_{50}	500 mg/m^3 or less	>500-2000 mg/m^3	>2000-200,000 mg/m^3
Oral, 14-day Single Dose LD_{50}	5 mg/kg or less	>5-50 mg/kg	>50-5000 mg/kg
Skin Absorption (Dermal) LD_{50}	20 mg/kg or less	>20-200 mg/kg	>200-20,000 mg/kg

During the current reporting period, a group of compounds was received and assigned code numbers prior to testing. These compounds, their code numbers and the studies performed on each are listed in Table 24.

In determining the oral toxicity of the above compounds, the following method was used. Water soluble compounds were administered in distilled water. All compounds which would not dissolve in water were given as suspensions in corn oil. All suspensions were kept in a turbulent state while dosing by stirring them on a magnetic stirring platform.

Glass syringes with special oral dosing needles were used to administer the compounds to the rats (200-300 grams). The experimental animals were fasted a minimum of 16 hours prior to the administration of the measured oral dose. This allows for more uniform

TABLE 24. LIST OF COMPOUNDS TESTED FOR ACUTE ORAL, INHALATION AND PERCUTANEOUS TOXICITY

Code No.	Compound	Route of Administration			
		Oral Tox.	Inhal. Tox.	Skin Absorp.	Skin Corros.
107	Perchloromethyl mercaptan		X	X	X
143	Boron trichloride		X		
144	Boron trifluoride		X		
165	Ethylchloroformate	X	X	X	X
170	Hexamethylene diamine	X	X	X	
180	Methylchloroformate	X	X	X	X
183	Red fuming nitric acid		X		
198	Fuming sulfuric acid		X		
248	n-Butyl acrylate	X	X	X	
249	Methyl acrylate		X		
250	Monoethanolamine	X			
251	Phenol, solid			X	X
252	Propionic acid (aqueous solution)				
	15% concentration				X
	1% concentration				X

TABLE 24. CONTINUED

Code No.	Compound	Route of Administration: Oral Tox.	Inhal. Tox.	Skin Absorp.	Skin Corros.
253	Hydrochloric acid (aqueous solution)				
	10% concentration				X
	5% concentration				X
	1% concentration				X
	0.1% concentration				X
	0.01% concentration				X
254	Sodium hydroxide (aqueous solution)				
	0.1% concentration				X
	0.01% concentration				X
255	Sulfuric acid (aqueous solution)				
	0.1% concentration				X
	0.01% concentration				X
256	Hydrofluoric acid (aqueous solution)				
	0.1% concentration				X
	0.01% concentration				X
257	Ethyl mercaptan		X		
258	Cresol (from coal tar) technical			X	X

TABLE 24. CONTINUED

Code No.	Compound	Route of Administration: Oral Tox.	Inhal. Tox.	Skin Absorp.	Skin Corros.
259	Cresol (from petroleum) technical			X	X
260	o-Cresol, practical			X	X
261	m-Cresol, practical			X	X
262	p-Cresol, practical			X	X
263	Sodium trichloro-s-triazinetrione	X		X	X
264	Fumaric acid	X		X	X
265	Maleic anhydride			X	X
266	Ammonium hydroxide				
	5% concentration				X
	1% concentration				X
267	Oxalic acid				
	5% concentration	X		X	X
268	Sodium sulfide (26% aqueous solution)				X
269	Sodium sulfhydrate (45% aqueous solution)				X
270	3-Methyl butyric acid			X	X
271	Tris-2-hydroxyethyliso-cyanurate	X		X	X

absorption in all the animals since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition. The injection volume used for the rats was 0.01 ml/gm which resulted in the average rat receiving a volume of 2.5 ml. The rats were weighed individually at the time of dosing to determine the proper injection volume. Geometrically spaced doses of the compounds were administered to the rats to determine the LD_{50}. Five rats were dosed at each level and the LD_{50} with its 95% confidence limits was calculated using the moving average interpolation method of Weil (1952).

Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally. Any rat which survived the 14-day postexposure observation period was sacrificed at that time.

For the dermal absorption toxicity determinations, female albino New Zealand rabbits (Pel-Freeze Bio-Animals, Inc.) weighing approximately 5 pounds were used as experimental animals. All rabbits were clipped as closely as possible with an Oster® clipper having surgical blades and a vacuum attachment. The backs of the rabbits were clipped from the saddle area of the shoulders to the top of the rear leg area and the sides down half way to the stomach area.

The rabbits were individually weighed to determine the proper dose volume. The measured quantity of the compound was then applied undiluted to the back of the rabbit and was divided equally between the two sides of the back. The dose was kept in place by applying 8-ply gauze patches over the liquid on each side of the back. A patch of latex rubber dental dam was then applied over the entire back area and elasto-plast tape was used to wrap the entire midsection of the rabbit to keep the gauze in place. Specially designed rabbit restraining harnesses (Newman, 1963) were fitted to each rabbit at the time of treatment. These harnesses restricted undesirable movement of the rabbits, i.e., prevented them from chewing on the taped area. The harnesses did, however, allow the rabbits complete freedom to eat and drink during the 24-hour contact period.

All dosing procedures were carried out in a fume hood due to the volatile nature of the compounds being tested. Rubber protective gloves were worn at all times by the personnel involved in the dosing procedures. The rabbits were housed in a hood during the 24-hour dosing period.

All compounds were applied undiluted. Solid materials were applied in coarse powder form and held in place with gauze patches and rubber dental dam. All test compounds remained in contact with the rabbit skin for 24 hours after which the gauze, tape and harness were removed. The rabbits were observed for signs of toxicity during the 14 days immediately following dosing.

Skin corrosion is defined as visible destruction or irreversible alteration of skin tissue after four hours contact. For the determination of skin corrosion all rabbits were clipped of all possible hair on the back and flanks 24 hours prior to contact to allow for recovery of the skin from any abrasion resulting from clipping. Six areas on the back, three per side, were designated as patch areas. This allowed for the simultaneous testing of six compounds on each rabbit.

The test material was applied in its native state, either solid or liquid, in the amount of 0.5 grams for solids or 0.5 ml for liquids. If the material was in the form of lumps, these were powdered prior to dosage. The compound was applied to the designated patch areas and then covered by a 1-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was then covered by a rubber dental dam strip and secured with the elastoplast tape. The patches remained in place

on the rabbits for 4 hours. The rabbits were fitted with leather restraining collars during the exposure period to prevent disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water.

After four hours, the wrap and gauze patches were carefully removed and the test areas evaluated for visible destruction or irreversible alteration in skin tissue. Tissue destruction does not include sloughing of the epidermis or erythema, edema or fissuring. Readings were also made at 48 and 72 hours (44 and 68 hours after the first reading).

The inhalation exposures for one-hour LC_{50} determinations on male and female rats were made in a 30-liter glass bell jar with an air flow of 30 liters/minute. Exposure groups consisted of 5 rats per contaminant level. The rats were observed for toxic signs during exposure and for the 14 days immediately following exposure. Animal weights were recorded immediately prior to exposure and again at 14 days post-exposure on survivors. LC_{50} determinations were calculated using the probit analysis method of Finney (1952).

If the compound had a low order of toxicity, a substantially saturated vapor was generated by bubbling dry air through a glass fritted disc immersed in the material. Groups of five rats were exposed to the saturated vapors in a 9-liter glass chamber for one hour. Solid compounds were tested in a static technique whereby the compound was

placed in a shallow, glass container and sealed into a 120 liter plexiglas chamber for a period of 24 hours. The rats were then rapidly introduced by means of a drawer-type cage designed to minimize vapor loss. The amount of compound necessary to saturate 120 liters of air was calculated from available vapor pressure data and then five times this amount was put into the chamber to ensure saturation. A small squirrel-cage blower was also in the chamber revolving at a slow speed to prevent layering and enhance mixing of the vapors. A container of Drierite® was also in the chamber to prevent reaction of the compound with moisture prior to insertion of the animals.

These studies are still in progress but completed work is tabulated for each type of test procedure.

One compound, tris-2-hydroxyethylisocyanurate, is of such a low order of toxicity that even a dose of 20 grams/kilogram, which is the maximum dose that can be tested was not lethal to male rats and only partially lethal to a group of 5 female rats.

A sex-related effect can be seen in the response of male and female rats to oral doses of ethyl and methylchloroformate. The female rats appear to be more susceptible to the compound by the oral route than the male rats. This response is the exact opposite of the sex-related

effect found with these two compounds by the inhalation route. The results of completed acute toxicity tests and the assigned classification (when applicable) are shown in Tables 25 through 28.

Only one compound, phenol, caused definite corrosion upon the clipped skin of rabbits. One half gram of phenol caused major necrosis over an area of 4 x 4 cm. None of the other compounds showed any corrosive effects when tested on five different rabbits.

Dynamic saturated vapor exposures were performed with the two acrylate compounds. Total mortality could not be achieved in either rat sex with vapors using these compounds during a one-hour exposure. Therefore, one-hour LC_{50}'s could not be calculated for these compounds.

A static saturated vapor exposure was conducted with the one solid compound (hexamethylene diamine). One-hour exposures of male and female rats had no visible toxic effects upon the animals. Rats were exposed to two concentrations of methyl mercaptan. Three of five female rats died at the highest concentration while no mortality occurred in the male rats. The high level concentration of methyl mercaptan is equivalent to the lower explosive limit of the compound; therefore, exposures were terminated at this point as it is obvious that the explosive hazard of this compound exceeds its acute toxicity potential.

TABLE 25. ORAL TOXICITY OF VARIOUS COMPOUNDS TO MALE AND FEMALE RATS

Compound	Sex	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
n-Butyl acrylate[a]	M	6169 (4567-8332)	4000 (0), 8000 (4)	Below toxic
n-Butyl acrylate[a]	F	4921 (4321-5604)	4000 (0), 5040 (3), 6350 (5)	Toxic
Hexamethylene diamine[b]	M	800 (472-1357)	400 (1), 800 (2), 1600 (5)	Toxic
Hexamethylene diamine[b]	F	746 (505-1104)	400 (0), 800 (3), 1600 (5)	Toxic
Monoethanolamine[b]	M	1970 (1431-2712)	800 (0), 1600 (1), 3200 (5)	Toxic
Monoethanolamine[b]	F	1715 (1159-2537)	800 (0), 1600 (2), 3200 (5)	Toxic
Ethylchloroformate[a]	M	467 (315-690)	250 (0), 500 (3), 1000 (5)	Toxic
Ethylchloroformate[a]	F	268 (181-396)	125 (0), 250 (2), 500 (5)	Toxic
Sodium trichloro-s-triazinetrione[c]	M	406 (295-559)	250 (0), 500 (4), 1000 (5)	Toxic
Sodium trichloro-s-triazinetrione[c]	F	466 (315-690)	250 (0), 500 (3), 1000 (5)	Toxic

TABLE 25. CONTINUED

Compound	Sex	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
Methylchloroformate[a]	M	187 (126-276)	100 (0), 200 (3), 400 (5)	Toxic
Methylchloroformate[a]	F	107 (73-159)	50 (0), 100 (2), 200 (5)	Toxic
Fumaric acid[a]	M	10,720 (7245-15,850)	5000 (0), 10,000 (2) 20,000 (5)	Below toxic
Fumaric acid[a]	F	9330 (6308-13,800)	5000 (0), 10,000 (3), 20,000 (5)	Below toxic
Oxalic acid 5%[b]	M	9.5 (5.4-12.3)[d]	4 (0), 8 (2), 16 (4), 32 (5)	Toxic
Oxalic acid 5%[b]	F	7.5 (5.0 - 11.0)[d]	4 (0), 8 (3), 16 (5), 32 (5)	Toxic
tris-2-hydroxyethyliso-cyanurate[b]	M	--	20,000 (0)	Below toxic
tris-2-hydroxyethyliso-cyanurate[b]	F	--	10,000 (0), 20,000 (3)	Below toxic

(a) = diluted in corn oil.

(b) = diluted in distilled water.

(c) = diluted in agar solution.

(d) = dosage given as ml/kg

TABLE 26. DERMAL TOXICITY OF COMPOUNDS TO FEMALE RABBITS

Compound	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 3)	Classification
n-Butyl acrylate	5657 (1451-22,050)	2000 (0), 4000 (1), 8000 (2)	Toxic
Hexamethylene diamine	1114 (600-2115)	625 (0), 1250 (2), 2500 (3)	Toxic
Phenol	1403 (739-2665)	625 (0), 1250 (1), 2500 (3)	Toxic

TABLE 27. CORROSIVE EFFECTS OF VARIOUS COMPOUNDS ON RABBIT SKIN

Compound	1	2	3	4	5	6	Remarks
Cresol (coal tar)	+	+	-	-	-	-	Definite corrosive
Cresol (petroleum)	+	o					
o-Cresol	+	+	-	-	-	-	Definite corrosive
m-Cresol	+	+	-	-	-	-	Definite corrosive
p-Cresol	+	+	-	-	-	-	Definite Corrosive
Ethylchloroformate	o	o	o	o	o	-	Noncorrosive
Sodium trichloro-s-triazinetrione	o	o	o	o	o	-	Noncorrosive
Fumaric acid	o	o	o	o	o	-	Noncorrosive
Perchloromethyl mercaptan	o	o					
Maleic anhydride	+	o	+	+	-	-	Definite corrosive
Ammonium hydroxide 5%	o	o	o	o	o	-	Noncorrosive
Ammonium hydroxide 1%	o	o	o	o	o	-	Noncorrosive
Oxalic acid 5%	o	o	o	o	o	-	Noncorrosive
Sodium sulfide 25-27% (aqueous solution)	+	+	-	-	-	-	Definite corrosive
Sodium sulfhydrate 45% (aqueous solution)	+	+	-	-	-	-	Definite corrosive
3-Methyl butyric acid	o	o	o	o	o	-	Noncorrosive
Tris-2-hydroxyethyl-isocyanurate	x	x	x	x	x	x	Noncorrosive
Methylchloroformate	o	o	o	o	o	-	Noncorrosive
Phenol	+	+	-	-	-	-	Definite corrosive
Propionic Acid 15%	o	o	o	o	o	-	Noncorrosive
Propionic Acid 1%	o	o	o	o	o	-	Noncorrosive
Hydrochloric Acid 10%	o	o	o	o	o	-	Noncorrosive
Hydrochloric Acid 5%	o	o	o	o	o	-	Noncorrosive
Hydrochloric Acid 1%	o	o	o	o	o	-	Noncorrosive
Hydrochloric Acid 0.1%	o	o	o	o	o	-	Noncorrosive

TABLE 27. CONTINUED

Compound	1	2	3	4	5	6	Remarks
Hydrochloric Acid 0.01%	o	o	o	o	o	-	Noncorrosive
Sodium Hydroxide 0.1%	o	o	o	o	o	-	Noncorrosive
Sodium Hydroxide 0.01%	o	o	o	o	o	-	Noncorrosive
Sulfuric Acid 0.1%	o	o	o	o	o	-	Noncorrosive
Sulfuric Acid 0.01%	o	o	o	o	o	-	Noncorrosive
Hydrofluoric Acid 0.1%	o	o	o	o	o	-	Noncorrosive
Hydrofluoric Acid 0.01%	o	o	o	o	o	-	Noncorrosive

+ = Caused visible destruction or irreversible alteration in skin tissue after 4 hours contact.

o = Did not cause visible destruction or irreversible alteration in skin tissue after 4 hours contact.

- = Not tested in that a positive of 2/6 or negative of 0/5 rabbits has already been produced.

x = Dosage of 20 g/kg caused no corrosive effects in dermal toxicity tests.

TABLE 28. ONE-HOUR INHALATION TOXICITY OF VARIOUS COMPOUNDS FOR MALE AND FEMALE RATS

Compound	Sex	LC_{50} (95% C. L.) in ppm	Data Used to Calculate LC_{50} in ppm (Mortality Response, N = 5)	Classification
n-Butyl acrylate	M	--	6360[a] (2)	--
n-Butyl acrylate	F	--	5100[a] (4)	--
Methyl acrylate	M	--	33,228[a] (1)	--
Methyl acrylate	F	--	34,315[a] (3)	--
Ethyl chloroformate	M		95 (0), 122 (2), 145 (5)	Highly toxic
Ethyl chloroformate	F		118 (0), 148 (1), 184 (4)	Highly toxic
Methyl chloroformate	M		59 (0), 79 (2), 100 (5), 121 (5)	Extremely toxic
Methyl chloroformate	F		77 (0), 97 (1), 128 (5)	Extremely toxic
Hexamethylene diamine	M	--	Substantially Saturated Vapor (0)	--
Hexamethylene diamine	F	--	Substantially Saturated Vapor (0)	--
Ethyl mercaptan	M	--	15,000 (0), 28,400[b] (0)	--
Ethyl mercaptan	F	--	15,000 (0), 27,700[b] (3)	--

[a] = Essentially saturated vapor concentration.

[b] = Explosive concentration.

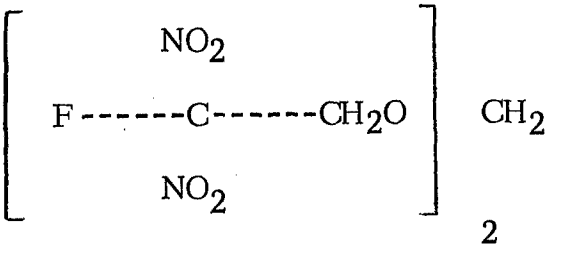
To calculate an LC_{50} by the Finney method, it is necessary to have a minimum of two concentration levels causing a partial mortality. Therefore, several exposures are needed to conclude work on the two chloroformate compounds to achieve data upon which an LC_{50} can be based. Inhalation exposures to boron trichloride and boron trifluoride are now in progress. The completion of these few inhalation exposures will conclude this DOT study.

Acute Toxicological Studies on Bis(2, 2-Dinitro-2-Fluorethoxy) Methane: FEFO

The compound Bis(2, 2-dinitro-2-fluorethoxy) methane, also designated FEFO or BDNEF, has a high potential for use both as a propellant constituent and as an explosive material. Previous acute toxicity studies (Barry et al., 1962), determined the mouse intraperitoneal LD_{50} and measured guinea pig skin and eye irritation. FEFO, diluted in corn oil, injected intraperitoneally into albino mice produced an LD_{50} of 90 mg/kg. The compound applied to the shaven skin of guinea pigs produced mild erythema after 24 hours which disappeared within 72 hours. A three percent solution in corn oil instilled into the eye of a guinea pig produced minimal lacrimation and irritation for approximately two hours.

To broaden the acute toxicity information on this compound, rats were exposed for six hours to substantially saturated vapors of the compound. In addition, male rats were dosed orally with the compound to establish a single dose LD_{50}.

The pertinent chemical and physical properties and structural formula are as follows:



Molecular weight	-	320
Density, g/ml	-	1.595 at 25 C
Boiling point	-	120° at 0.3 mm Hg
Freezing point	-	14 C

FEFO was administered orally to rats as a suspension in corn oil which was kept in a turbulent state while in use by stirring on a magnetic stirring platform. The oral dosing technique was the same as that described in previous sections of this report. Five rats were dosed at each concentration level and the LD_{50} calculated using the moving average interpolation method of Weil (1952).

Deaths which occurred during the 14 days immediately following the administration of the single oral dose were included in the final mortality tally. All that survived the 14-day postexposure observation period were sacrificed at that time.

Five male rats were exposed to essentially saturated vapors of the compound in a static technique whereby the compound was placed in a large watch glass and inserted into a 120 liter sealed chamber for 24 hours. The rats were then rapidly introduced by means of a drawer-type cage designed to minimize vapor loss.

The amount of compound necessary to saturate 120 liters of air was calculated from vapor pressure data, then five times that amount was put into the chamber to ensure saturation. The chamber contained a small squirrel cage blower running at a slow speed which prevented layering of the vapors. A pan with Drierite® was also put into the chamber to prevent reaction of the compound with moisture prior to insertion of the animals.

Gross and histopathological examinations were performed on any rat that died following the administration of the oral dosing. Rats that survived the 14-day postexposure observation period were only examined grossly. The results of dosing are shown in Table 29.

TABLE 29. ACUTE SINGLE DOSE ORAL TOXICITY OF BIS(2,2-DINITRO-2-FLUORETHOXY) METHANE: FEFO

Dose, mg/kg	Mortality Ratio*
2000	5/5
1588	5/5
1260	5/5
1000	0/5

LD_{50} = 1122 (range 1000-1260) mg/kg

*Number that died over the number dosed.

FEFO does not appear to be very toxic by the peroral route. The dose-mortality response curve is very steep, however, and a partial mortality was not obtained. The lack of a partial mortality level precluded calculation of the 95% confidence limits so, instead, a range is included with the LD_{50}.

Many of the rats which died following oral doses of FEFO showed distended stomachs upon gross examination with bleaching of the greater curvature. Often this was accompanied by bleaching of the liver lobes which were in close contact to the stomach. Histopathological examination of these animals showed lesions of acute necrotizing gastritis, enteritis and splenitis.

Five male rats, exposed for six hours to essentially saturated vapors of FEFO, survived the exposure with no visible signs of toxic stress either during exposure or in the 14-day observation period. Four of the five rats showed normal weight gains during the 14-day period. At necropsy, two had slightly mottled kidneys while the other three appeared essentially normal.

The results of these tests indicate that FEFO has a low toxicity. FEFO vapors generated at room temperature under ambient conditions do not constitute a hazard to man provided the exposure does not continue for prolonged time periods.

THRU REPORT 1975

Studies on the Effect of Monomethylhydrazine in Drinking Water on Golden Syrian Hamsters

Recent studies by Toth (1972, 1973) have shown hydrazines to have carcinogenic activity when administered continuously in the drinking water of Swiss mice and Golden Syrian hamsters. In the first of these studies, solutions of 0.001% hydrazine, 0.01% methylhydrazine and 0.001% methylhydrazine sulfate were given daily ad libitum to 5 and 6 week old randomly bred Swiss mice for their entire lifetimes. Hydrazine and methylhydrazine sulfate significantly increased the incidence of lung tumors in the mice, while methylhydrazine enhanced the development

of neoplasms by shortening the latent period. In the second of Toth's studies, Golden Syrianhamsters received 0.01% methylhydrazine in drinking water daily ad libitum for life. Malignant histiocytomas (Kupffer cell sarcomas) were observed in the livers of 54% of the male hamsters treated, while none were observed in the control groups.

These investigations indicate that MMH is carcinogenic in animals and therefore, may pose a formidable hazard to man. However, two areas of concern prompted further review of these findings: (1) the calculated daily dose of MMH received by the experimental animals had been shown to produce acute toxic effects when given to rodents and other animal species by inhalation or injection routes of administration, and (2) other studies had shown that MMH was very unstable in the presence of oxygen and it was, therefore, probable that the animals were not exposed to MMH (at least not a 0.01% solution) but to its oxidation products which are generally considered nontoxic.

The investigation completed this year was designed to test the reproducibility of the carcinogenic activity of MMH administered in the drinking water of male Golden Syrian hamsters.

Preliminary experiments to determine the stability of a 0.01% MMH-tap water solution stored in feeder bottles demonstrated a loss of approximately 60% of the MMH content over a 24-hour period. It was found that the pH of the MMH-water solution had a great effect on decomposition. A solution of MMH and tap water adjusted to a pH of 3.5 with HCl was found to retain approximately 95% of the MMH content over a 24-hour period. Other preliminary experiments showed that drinking water adjusted to a pH 3.5 with HCl, and fed to hamsters, did not influence body weight gain or water consumption when compared with untreated tap water.

The three groups of hamsters and drinking solutions used to investigate the carcinogenic effect of MMH were as follows:

Control - drinking water adjusted to pH 3.5 with HCl

Buffered MMH Solution - 0.01% MMH in drinking water adjusted to pH 3.5 with HCl

Unbuffered MMH Solution - 0.01% MMH in drinking water not pH adjusted.

Each of the groups receiving MMH consisted of 30 hamsters. Controls numbered 17. At the beginning of the study, the hamsters were approximately 5 months old.

All animals were gang caged, with five hamsters per cage, and supplied lab chow ad libitum. Drinking solutions were prepared daily utilizing a 5-liter glass volumetric flask and individual premeasured aliquots of MMH and HCl contained in glass vials. Water solutions were changed daily.

Test and control hamsters were weighed on a monthly schedule. Water consumption measurements were made for the 24-hour period preceding a scheduled weighing to allow for calculation of the nominal MMH dose ingested on a daily basis.

For the first 11 months of the experiment, the nominal average daily dose of MMH was calculated to be 7.3 mg/kg body weight/day for the group receiving unbuffered MMH, and 7.5 mg/kg body weight/day for the group receiving buffered MMH solution. These average daily dose data are nominal calculations and do not consider any degradation of MMH solution which takes place in the feeder bottles.

Maximum growth of the hamsters used in this study had occurred by the fourth month of MMH treatment since the animals were approximately 5 months old at the beginning of the test period. The mean weight of hamsters receiving the unbuffered MMH solution paralleled the control group mean body weight until the 15th month when weight losses occurred

as shown in Figure 7. The group of hamsters receiving the buffered MMH solution had significantly lower mean body weights than the control group throughout the study after the third month of treatment. After the eleventh month of the study, when the hamsters were approximately sixteen months old, all groups exhibited a gradual but steady loss of weight.

To determine hemolytic effects of MMH during the course of the study, hematocrit and red blood cell measurements were made on blood samples taken by orbital puncture from 5 hamsters in each experimental group at 7, 11 and 15 months. Results are shown in Table 30. Depressed hematology values, evidence of RBC destruction is clearly demonstrated for both groups of MMH dosed animals.

TABLE 30. HEMATOLOGIC EFFECTS IN HAMSTERS INDUCED BY 0.01% MMH IN DRINKING WATER

Months of Study	Control		Unbuffered MMH		Buffered MMH	
	RBC*	HCT**	RBC	HCT	RBC	HCT
7	8.74	52	6.65	44	6.77	45
11	8.45	53	7.05	45	6.17	45
15	7.95	49	6.04	40	6.32	43

* RBC values in millions.

**HCT values in percent.

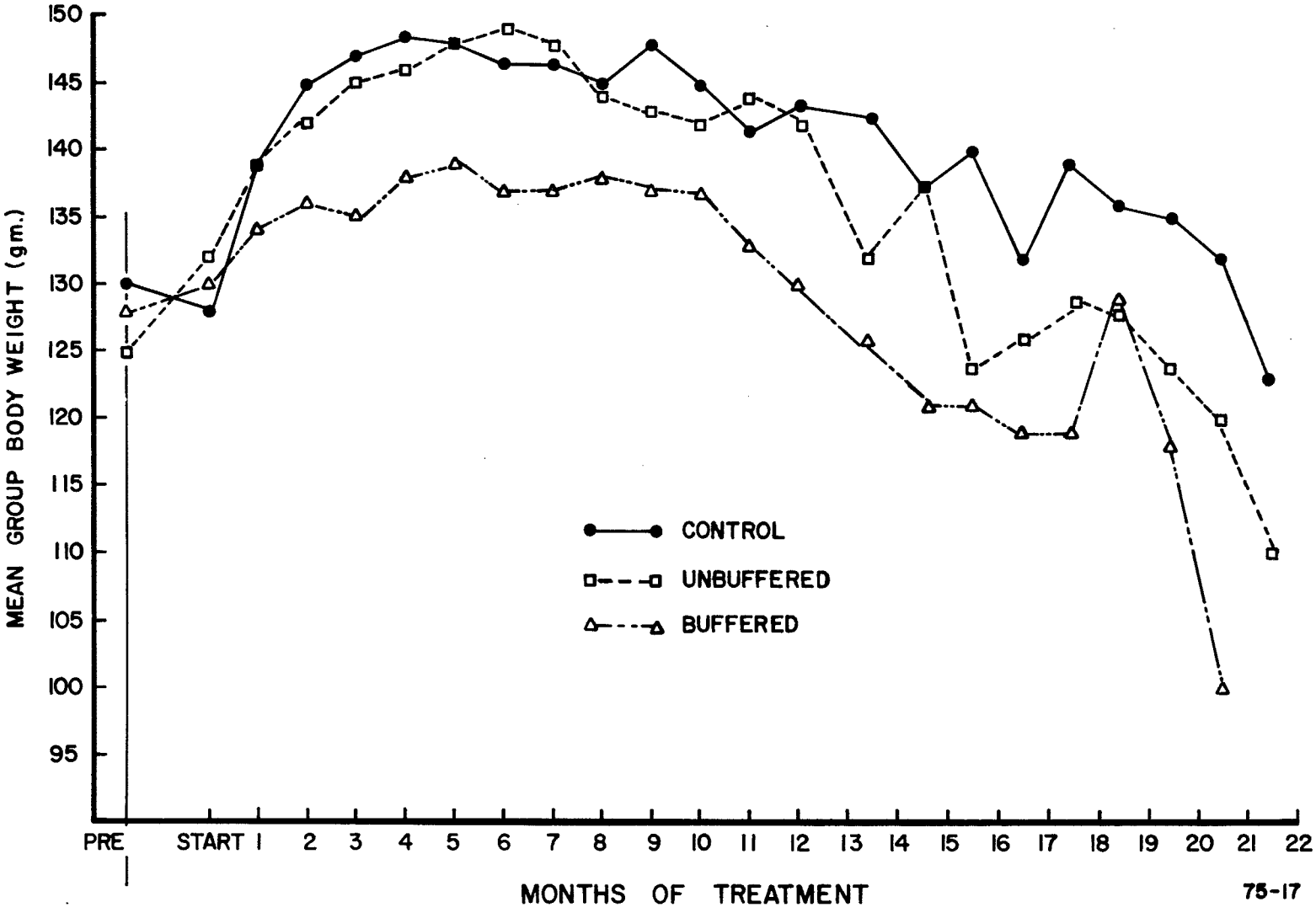


Figure 7. The effect of drinking water containing monomethylhydrazine on Golden Syrian hamster growth.

Further measurements of hematological parameters and examination of bone marrow were made at the conclusion of the study on 2 survivors from the buffered group and one control. As seen previously, hematology values for MMH dosed hamsters were significantly lower than the control. Increased erythroid production was demonstrated in myeloid-erythroid determinations made on the hamsters used in this study. The control animal had a M/E ratio of 1.9 compared to 0.5 and 0.7 for the 2 hamsters drinking 0.01% MMH solution buffered to pH 3.5 for 83 weeks.

All experimental animals were maintained for their lifetimes on the drinking solutions under study. Hamster survival rates and length of treatment are depicted in Table 31. The most common cause of death in hamsters from all groups in this study was renal insufficiency due to severe amyloidosis, a condition that was seen in almost every animal.

Animals were given a complete necropsy following death or sacrifice at termination of the study. Histologic examinations were performed on tissues from the lung, heart, esophagus, trachea, thyroid, liver, spleen, kidney, urocyst and testes plus any lesions seen at necropsy. The number of animals subjected to histologic examination

TABLE 31. SURVIVAL RATE OF MMH TREATED AND CONTROL HAMSTERS

Weeks of Treatment	Percentage of Survivors		
	Control	Buffered MMH	Unbuffered MMH
10	100%	100%	100%
20	100	100	100
30	100	100	100
40	100	100	93
50	94	97	80
60	64	70	77
70	52	43	47
80	24	17	17
90	12	0*	3

*Two remaining survivors were moribund and were killed at 83 weeks.

are as follows: Control - 13, Buffered - 25, Unbuffered - 25. The remaining animals from each group were unsuitable for histologic examination at death either due to severe autolysis or cannibalism of critical tissues.

Table 32 lists the number and types of neoplasms found in this study. The total was 14 neoplasms with adrenocortical tumors predominating (2 adenomas and 7 carcinomas). Spontaneous occurrence of adrenal neoplasms is a common finding in aged hamsters with as high as 35% incidence reported in animals between 2 and 3 years of age. Three liver tumors (one hemangioendothelioma and two hepatocellular carcinomas) were found in hamsters from the unbuffered group and 2 skin tumors (one histiocytoma and one melanoma) were found in hamsters receiving unbuffered MMH.

Neither the incidence, degree of severity, nor age of onset of nonneoplastic pathologic changes was markedly different between animals drinking MMH in water and control animals. The presence of 23% incidence of adrenocortical tumors in control animals versus 4% in Group I (MMH in tap water) and 12% in Group II (MMH + pH 3.5 water) argues against MMH as a cause of these tumors. The five remaining neoplasms, one hemangioendothelioma of the liver, two hepatocellular carcinomas, one cutaneous histiocytoma and one cutaneous melanoma, occurred only

TABLE 32. NEOPLASMS FOUND IN HAMSTERS RECEIVING 0.01% MMH IN DRINKING WATER

Animal Group	Neoplasms
Control (pH 3.5 water) N = 17	a) Adenoma, adrenal cortex b) Adenoma, adrenal cortex (left adrenal); Carcinoma, adrenal cortex (right adrenal) c) Carcinoma, adrenal cortex TOTAL TUMORS = 4
Unbuffered (MMH + water) N = 30	a) Carcinoma, adrenal cortex, metastatic to lung b) Hemangioendothelioma of liver c) Hepatocellular carcinoma d) Hepatocellular carcinoma TOTAL TUMORS = 4
Buffered (MMH + pH 3.5 water) N = 30	a) Carcinoma, adrenal cortex b) Carcinoma, adrenal cortex, metastatic to lung c) Carcinoma, adrenal cortex, bilateral d) Histiocytoma, skin of thorax e) Melanoma, skin of ear TOTAL TUMORS = 6

in the experimental groups. They were derived from four different cell types and as such constitute a 4% incidence for each tumor in their respective group of animals, except for an 8% incidence of hepatocellular carcinoma. The overall tumor incidence for Group I (MMH + tap water) was 16%, Group II (MMH + pH 3.5 water) was 24%, and Group III (control) was 31%. These findings are in contrast to the findings of Toth and Shimizu (1973). They reported a 54% incidence of Kupffer cell sarcomas, a 14% incidence of cecal tumors, and an overall tumor incidence of 101% in 50 male hamsters. Ninety-nine controls had an overall tumor rate of 27% with no Kupffer cell sarcomas and 1 cecal tumor reported. The dose of MMH was 0.01% administered in drinking water for the lifetime of the animals.

The small numbers of animals involved in both laboratory studies plus the absence of a high incidence of neoplasia in our experimental groups precludes speculation on the oncogenicity of monomethylhydrazine administered at 0.01% in water ad lib for life to hamsters, but our results are in essence negative.

90-Day Continuous Coal Tar Aerosol Inhalation Studies

Animals from three 90-day continuous coal tar aerosol studies (10, 2, and 0.2 mg/m^3 coal tar) were maintained during the past year. The description of the coal tar generation method analysis and experiment protocols is given in the previous annual report (MacEwen and Vernot, 1974). During the postexposure holding period, the rabbits were bled and all animals weighed on a monthly schedule. All animals were also examined biweekly for the development of skin lesions.

Changes from the original protocol were made during this time period due to two epizootic outbreaks in the animal holding facilities. The number of weanling rats was seriously depleted due to an infectious respiratory disease. Many CF-1 mice died as a result of a type G streptococcus infection which appeared to be spread by biting. As a result of this, blood sampling of the weanling rats and serial sacrifice of CF-1 mice was terminated in each of the studies. The serial sacrifices of the CF-1 mice were terminated in June, 1974 which was 11, 8 and 4 months postexposure for the 10, 2 and 0.2 mg/m^3 exposure groups, respectively. The bleeding of weanling rats concluded in November, 1974 which was at 16, 13 and 9 months postexposure for the respective studies.

Summaries of the mouse skin tumors found after the 90-day exposure to 10 and 2 mg/m^3 coal tar aerosol are shown in Tables 33 through 36. Beginning at 6 weeks postexposure, the animals were examined biweekly. Mouse skin tumors were found during the first inspection of both the 10 and 2 mg/m^3 groups. No skin tumors have been found in the mice exposed to 0.2 mg/m^3 after 61 weeks of postexposure observation.

More than 75% of the total number of skin tumors in the CF-1 mice exposed to 10 mg/m^3 were found between 14 and 35 weeks postexposure. Seventy-nine of a total of 102 skin tumors developed during this 11 week span. Of the 23 skin tumors found thus far in the CF-1 mice exposed to 2 mg/m^3 coal tar, 18 or 78% of these were found during the first 22 weeks of postexposure observation.

The following tabulation, showing the cumulative number of skin tumors that developed after a comparative postexposure period for all three studies, demonstrates a definite dose-response effect. This effect is strongly apparent in the CF-1 mice but less obvious in the JAX (CAF-1) mice where smaller number of skin tumors arose. However, when comparing the JAX mice data from a later period (Tables 33-36), it can be seen that the number of tumors increased during the later stages of the postexposure holding period. It appears that the JAX mice have a much longer period of onset than the CF-1 strain for development of skin tumors.

TABLE 33. SUMMARY OF SKIN TUMORS FOUND IN CF-1 MICE EXPOSED TO 10 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	New Tumors		Cum. No. of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
6	3	0	3	0	55	109
8	0	3	3	3	53	109
10	0	0	3	3	52	98
12	5	0	8	3	52	94
14	0	0	8	3	52	88
17	0	15	8	18	52	87
21	12	9	20	27	49	67
23	7	8	27	35	47	63
25	2	2	29	37	47	63
27	4*	3	33(25)	40	47	63
29	2	4	35(26)	44	45	58
31	3	1	38(28)	45	41	55
33	0	4	38(25)	49(35)	39	53
35	0	3	38(24)	52(29)	39	43
37	1	1	39(23)	53(29)	37	39
39	0	1	39(22)	54(26)	33	35
41	0	2	39(20)	56(22)	28	28
43	2*	1*	41(19)	57(20)	23	24
45	0	0	43(16)	58(18)	20	22
47	0	0	43(13)	58(15)	17	19
49	0	0	43(11)	58(12)	15	14
51	0	0	43(10)	58(11)	12	12
53	1	0*	44(11)	58(4)	11	4
55	0	0	44(4)	58(2)	4	2
57	0	0	44(4)	58(1)	4	1
59	0	0	44(3)	58(1)	3	1
61	0	0	44(3)	58(0)	3	0
63	0	0	44(3)	58(0)	3	0
65	0	0	44(3)	58(0)	3	0
67	0*	0	44(1)	58(0)	1	0
69	0	0	44(1)	58(0)	1	0

TABLE 33. CONTINUED

Number of Weeks Postexposure	New Tumors		Cum. No. of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
71	0	0	44(1)	58(0)	1	0
73	0	0	44(1)	58(0)	1	0
75	0	0	44(0)	58(0)	0	0
77	0	0	44(0)	58(0)	0	0
79	0	0	44(0)	58(0)	0	0
81	0	0	44(0)	58(0)	0	0
83	0	0	44(0)	58(0)	0	0
85	0	0	44(0)	58(0)	0	0
87	0	0	44(0)	58(0)	0	0

Group A = Unclipped mice (original N = 75).

Group B = Clipped mice for pulmonary pathology (original N = 150).

( ) = Number of mice with tumors which are alive at this date.

* A control CF-1 mouse was found with a tumor on this date.

TABLE 34. SUMMARY OF SKIN TUMORS FOUND IN JAX MICE EXPOSED TO 10 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	New Tumors	Cumulative Number of Tumors	Total Examined
6	0	0	43
8	0	0	43
10	0	0	43
12	0	0	43
14	0	0	43
17	0	0	42
21	0	0	42
23	1	1(1)	42
25	0	1(1)	42
27	1	2(2)	41
29	1	3(3)	41
31	0	3(3)	41
33	0	3(3)	41
35	0	3(3)	41
37	0	3(3)	41
39	0	3(3)	41
41	1	4(2)	38
43	1	5(3)	38
45	0	5(3)	37
47	0	5(3)	37
49	0	5(3)	37
51	2	7(5)	35
53	3	9(8)	35
55	0	9(3)	29
57	0	9(2)	26
59	0	9(2)	24
61	0	9(2)	22
63	0	9(2)	22
65	0	9(2)	22
67	4	13(5)	20
69	0	13(3)	16

TABLE 34. CONTINUED

Postexposure	New Tumors	Cumulative Number of Tumors	Total Examined
71	2	15(5)	15
73	1	16(4)	12
75	0	16(2)	9
77	2*	18(3)	8
79	0	18(3)	8
81	0	18(3)	8
83	0	18(3)	8
85	0	18(2)	7
87	0	18(2)	6

* A control CF-1 mouse was found with a tumor on this date.

( ) = Number of mice with tumors which are alive at this date.

TABLE 35. SUMMARY OF SKIN TUMORS FOUND IN CF-1 MICE EXPOSED TO 2 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	New Tumors		Cum. No. of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
6	3	2	3	2	75	124
8	1	0	4	2	75	123
10	0	3	4	5	75	120
12	0	1	4(4)	6	75	94
14	0	0	4(4)	6	74	82
16	0	0	4(4)	6	69	73
18	1	0	5(5)	6(4)	69	54
20	0	3	5(4)	9(6)	66	46
22	4	0	9(8)	9(4)	66	37
24	0	0	9(7)	9(4)	61	31
26	0	0	9(7)	9(2)	60	25
28	0	1	9(7)	10(2)	58	24
30	1	0*	10(7)	10(1)	58	23
32	0	0	10(7)	10(1)	48	18
34	0	0	10(7)	10(1)	45	16
36	0	0	10(7)	10(1)	45	16
38	0	0	10(7)	10(1)	44	10
40	0	0	10(7)	10(0)	44	8
42	2	0	12(9)	10(0)	44	8
44	0	0	12(9)	10(0)	44	7
46	0	0	12(7)	10(0)	44	7
48	0	0	12(7)	10(0)	43	7
50	0	0	12(7)	10(0)	43	6
52	0	0	12(7)	10(0)	43	6
54	0	0	12(7)	10(0)	43	6
56	0	0	12(7)	10(0)	43	6
58	0	0	12(6)	10(0)	40	5
60	0	0	12(6)	10(0)	40	5
62	1	0	13(6)	10(0)	37	5
64	0	0	13(6)	10(0)	35	5
66	0	0	13(5)	10(0)	33	3
68	0	0	13(5)	10(0)	33	3

TABLE 35. CONTINUED

Number of Weeks Postexposure	New Tumors		Cum. No. of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
70	0	0	13(5)	10(0)	32	1
72	0	0	13(5)	10(0)	31	1
74	0	0	13(4)	10(0)	29	1
76	0	0	13(4)	10(0)	29	1
78	0	0	13(4)	10(0)	27	1
80	0	0	13(2)	10(0)	24	1
82	0	0	13(2)	10(0)	22	0
84	0	0	13(2)	10(0)	22	0

Group A = Unclipped mice (original N = 75).

Group B = Clipped mice for pulmonary pathology (original N = 150).

( ) = Number of mice with tumors which are alive on this date.

A A control mouse was found with a skin tumor on this date.

TABLE 36. SUMMARY OF SKIN TUMORS FOUND IN JAX MICE EXPOSED TO 2 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	New Tumors	Cumulative Number of Tumors	Total Examined
6	0	0	65
8	0	0	65
10	0	0	65
12	0	0	64
14	0	0	62
16	1	1(1)	59
18	0	1(1)	59
20	0	1(1)	54
22	2	3(3)	54
24	0	3(3)	51
26	0	3(2)	50
28	0	3(2)	47
30	0	3(2)	47
32	0	3(2)	44
34	0	3(2)	44
36	0	3(1)	40
38	0	3(1)	39
40	0	3(1)	36
42	0	3(1)	36
44	0	3(1)	36
46	0	3(1)	31
48	0	3(1)	31
50	0	3(1)	31
52	0	3(1)	31
54	0	3(1)	31
56	0	3(1)	31
58	0	3(0)	30
60	0	3(0)	30
62	0	3(0)	30
64	0	3(0)	30
66	0	3(0)	30
68	0	3(0)	30
70	0	3(0)	29
72	0	3(0)	28
74	0	3(0)	27
76	0	3(0)	24
78	0	3(0)	24
80	0	3(0)	23
82	0	3(0)	22
84	0	3(0)	22

( ) = Number of mice with tumors which are alive on this date.

Expos. Conc. mg/m^3	Weeks	Cumulative Number of Tumors		
		ICR Mice		
		Group A	Group B	JAX Mice
10	61	44	58	9
2	62	13	10	3
0.2	61	0	0	0

To date, the only other species to develop skin tumors is the hamster. Seven skin tumors were found in this species, all in the group exposed to 10 mg/m^3 coal tar aerosol. Grossly, these tumors resemble the skin tumors of the mice. A description of the tumors is detailed in the last annual report.

The 10 mg/m^3 study was terminated after a 20-month post-exposure period. The surviving animals were sacrificed and representative tissues were sampled for histopathological examination. Histopathology of all animals in these studies has been contracted to another source and the results are not yet available.

An 18-Month Inhalation Exposure of Animals to Coal Tar Aerosol

A long-term industrial-type inhalation exposure of four animal species to the complete complex mixture of coal tar volatiles was initiated for comparison with the 90-day continuous studies to validate the experimental approach of time compression with continuous animal exposures. This study will also examine the effects of inhaled coal tar aerosols on Macaca mulatta, a species more closely related to man.

Animals are being exposed intermittently (6 hours per day) on regular work days over a 18-month period to a 10 mg/m^3 concentration of coal tar aerosol. The exposures are being made in two Thomas Domes with a control group of animals being housed in the animal holding facilities for comparison with test groups. The animals are observed daily for general appearance, behavior, signs of toxic stress, morbidity and mortality.

Experimental animals include male and female CFE weanling rats, female CF-1 mice, male CAF-1 mice, female New Zealand albino rabbits, and male and female Macaca mulatta monkeys. (The CAF-1 mice were added one month after the start of exposures.) The animal complement and chamber loads are as follows:

1. Test Animals

Dome 2

18 New Zealand albino rabbits (female)

80 CFE weanling rats (40 male, 40 female)

Dome 3

14 Macaca mulatta monkeys (5 male, 9 female)

75 CF-1 mice (female) - Group A

100 CF-1 mice (female - toes clipped) - Group B

50 CAF-1 mice (male)

2. Control Animals

18 New Zealand albino rabbits (female)

80 CFE weanling rats (40 male, 40 female)

14 Macaca mulatta monkeys (5 male, 9 female)

75 CF-1 mice (female) - Group A

100 CF-1 mice (female - toes clipped) - Group B

50 CAF-1 mice (male)

Body weights of the individual rats were recorded immediately prior to onset of exposure at biweekly intervals during the first four months of exposure and monthly thereafter. Monkeys and rabbits were weighed immediately preexposure and at monthly intervals throughout the study.

Complete hematology with indices is being conducted on rabbits on a monthly basis during and after exposure for comparison with previous studies. Monkeys are sampled on a monthly basis (during and after exposure) for complete hematology studies and for the following battery of clinical chemistry determinations:

Sodium	Chloride	Total Protein	Alkaline Phosphatase
Potassium	Glucose	SGOT	
Calcium	Albumin	SGPT	Total Inorganic Phosphorus

After one and seven days, five exposed and five control Group B mice were sacrificed for gross and histological examination, particularly for pulmonary pathology. Four mice from Group B were examined bimonthly thereafter. The Group B mice were numbered and are sacrificed from a random number list. In the event of spontaneous deaths, the next number is selected. These mice also have a 1 x 1 inch patch of skin (fur intact) removed from their backs and a section of lung submitted to the chemistry department for analysis of fluorescent compounds.

Ten percent of the exposed and control rat groups will be sacrificed at the termination of exposures. The remaining animals will be observed a minimum of 12 months before being sacrificed. At that time, a decision will be made regarding disposition of the primate group. All

animals that die will be necropsied and tissues harvested for histological examination. Tissues to be sampled for histology include liver, lung genito-urinary tract (including bladder), scrotum, skin, spleen, larynx, nasal area and bone marrow.

Six months after the initiation of the study, routine skin tumor examinations were begun. The mice are examined biweekly while all other species are being examined monthly at the scheduled weighing period.

The aerosol generation units for this study are identical to those used in the previous 90-day studies. The technique for this aerosol generation system is described in detail in the 1973 annual report (MacEwen and Vernot, 1973).

The chamber coal tar aerosol concentrations are analyzed using gravimetric sampling to trap the aerosol droplets on a millipore filter. The fluorescent materials are dissolved from the filter with toluene and the fluorescence measured by a Turner fluorometer. Sampling is being done hourly during the exposure period. Each chamber was sampled daily for benzene concentration during the first month of exposure. The mean concentration of benzene was less than 10 mg/m^3 for either dome and a benzene hazard did not exist as

this concentration was well below the TLV of 80 mg/m^3. A monthly spot check of benzene concentrations is being done during the remaining portion of the study.

Benzene measurements were also made at one-half hour after the termination of the daily exposure with negative results. The lower limit of detection for the benzene analysis is approximately 2 mg/m^3.

An aerosol particle size determination is made once per month during the study following the procedure of Vooren and Meyer (1971). Table 37 shows the results of the monthly droplet size determinations. A minimum of 99% of the total droplets in both chambers has been five microns or less in diameter. It is, therefore, safe to presume that all aerosol droplets within the chambers are of a respirable size.

During the course of the study, rat, monkey and rabbit weights are monitored on a regularly scheduled basis. Figures 8 and 9 show the mean body weight relationship of the test animals with their respective controls. Because the rabbits originally scheduled to enter this experiment did not pass our standard quality control specifications, a second group had to be ordered. These entered the experiment two months after initiation; therefore, the rabbit mean body weights are two months behind the rats and monkeys.

TABLE 37. MEAN COAL TAR AEROSOL DROPLET SIZE DURING ANIMAL INHALATION EXPOSURES TO A 10 MG/M^3 CONCENTRATION

Month of Exposure	Percentage of Droplets 5 Microns or Less in Diameter	
	Dome 2	Dome 3
1	99.7	100
2	100	99.5
3	99.7	100
4	99.9	100
5	99.8	100
6	99.4	100
7	100	100

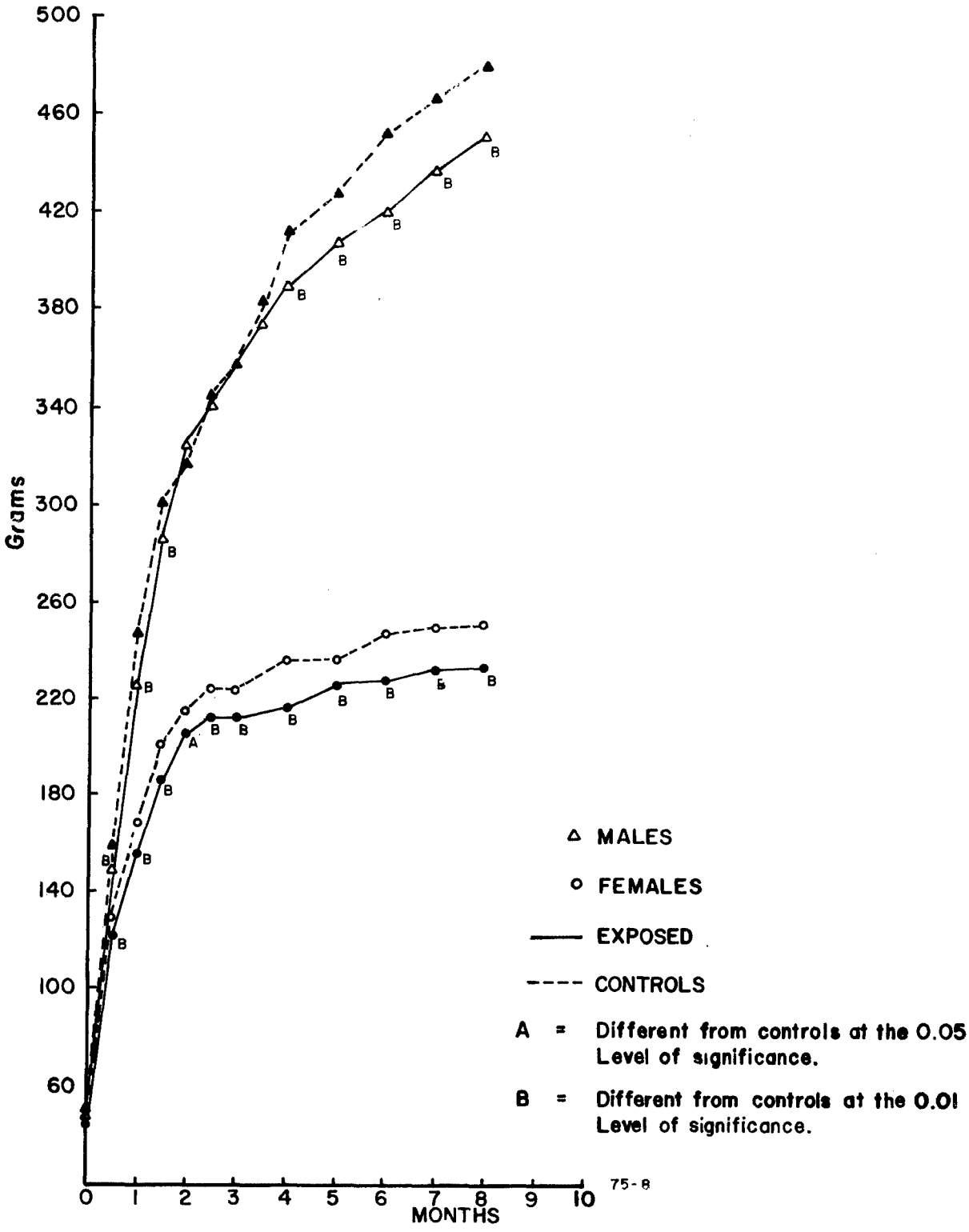


Figure 8. Effect of 18-month intermittent exposure to 10 mg/m^3 coal tar aerosol on growth of weanling rats.

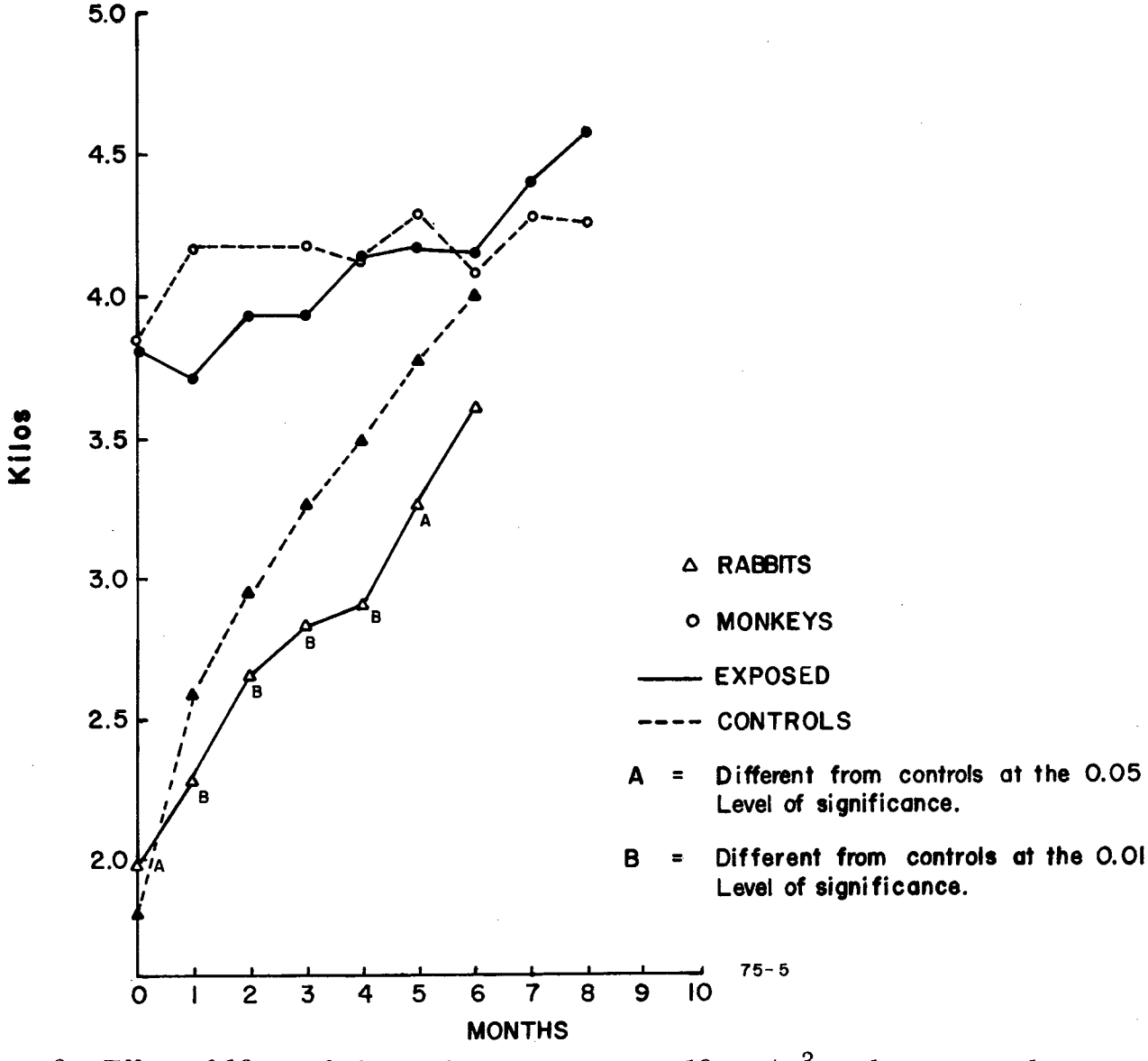


Figure 9. Effect of 18-month intermittent exposure to 10 mg/m^3 coal tar aerosol on growth of rabbits and monkeys.

Except for a brief six-week period, during which weights of control male rats dropped for some unknown reason, all exposed rats showed a statistically significant depression in mean body weights. This was apparent from the first weighing after the start of the study, at two weeks, and on through eight months of exposure. The rabbits show a similar pattern with the exposed rabbits having a statistically significant weight depression.

Eleven test and four control rabbits died after the study began. This mortality has been attributed to a chronic respiratory infection which caused severe debilitation and dehydration resulting, eventually, in death. The surviving rabbits show a depression in mean body weight similar to that demonstrated by the rats. In every case, the difference in the mean weights of the experimental animals is statistically significant when compared with their respective controls.

Several electrolyte values of exposed monkeys have shown a continually significant increase over control values during the course of the study. Potassium, calcium and glucose values in the test group showed an increase after two weeks of exposure and have continued in this manner throughout the study (Figures 10-12).

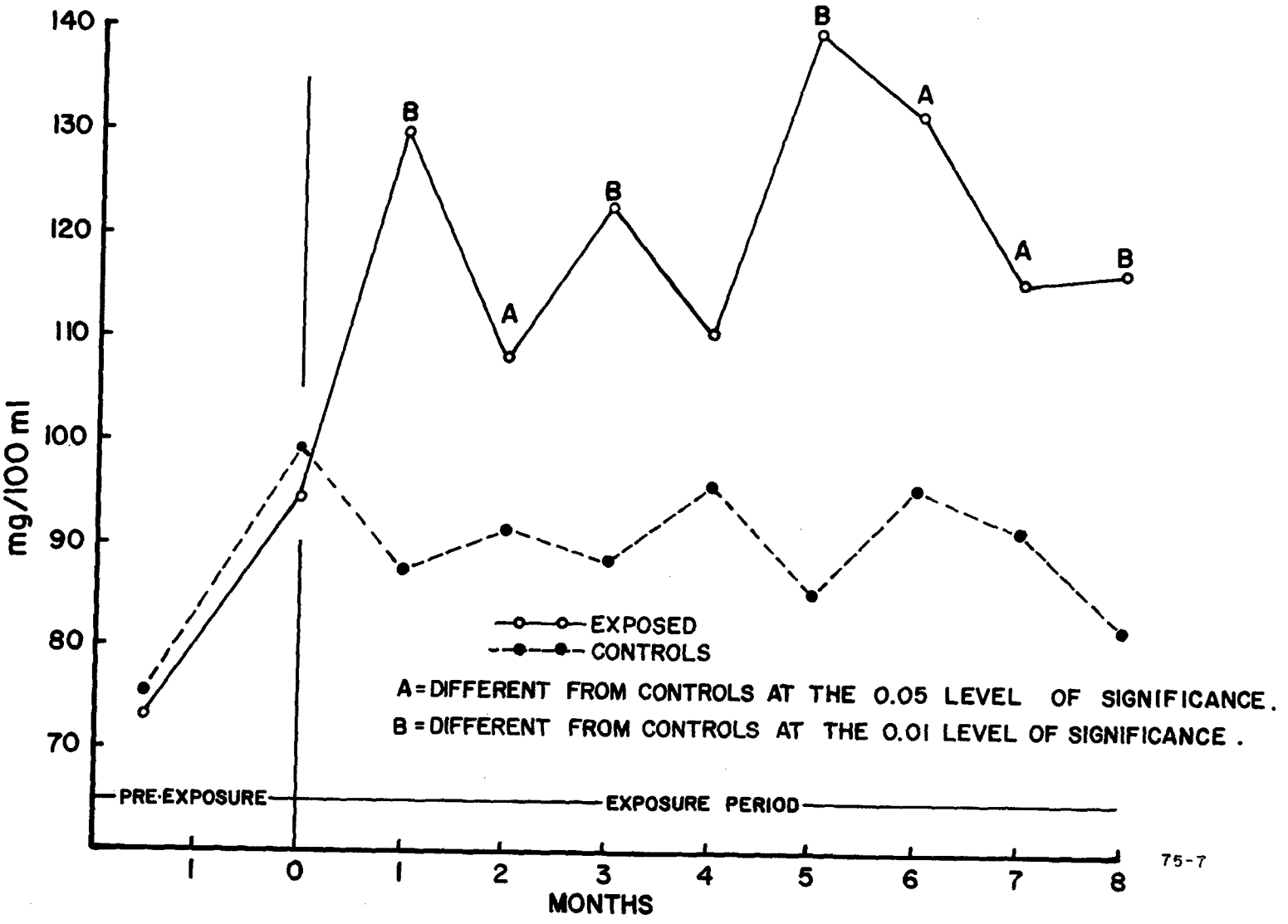


Figure 10. Mean glucose values in monkeys exposed to 10 mg/m^3 coal tar aerosol (N = 14).

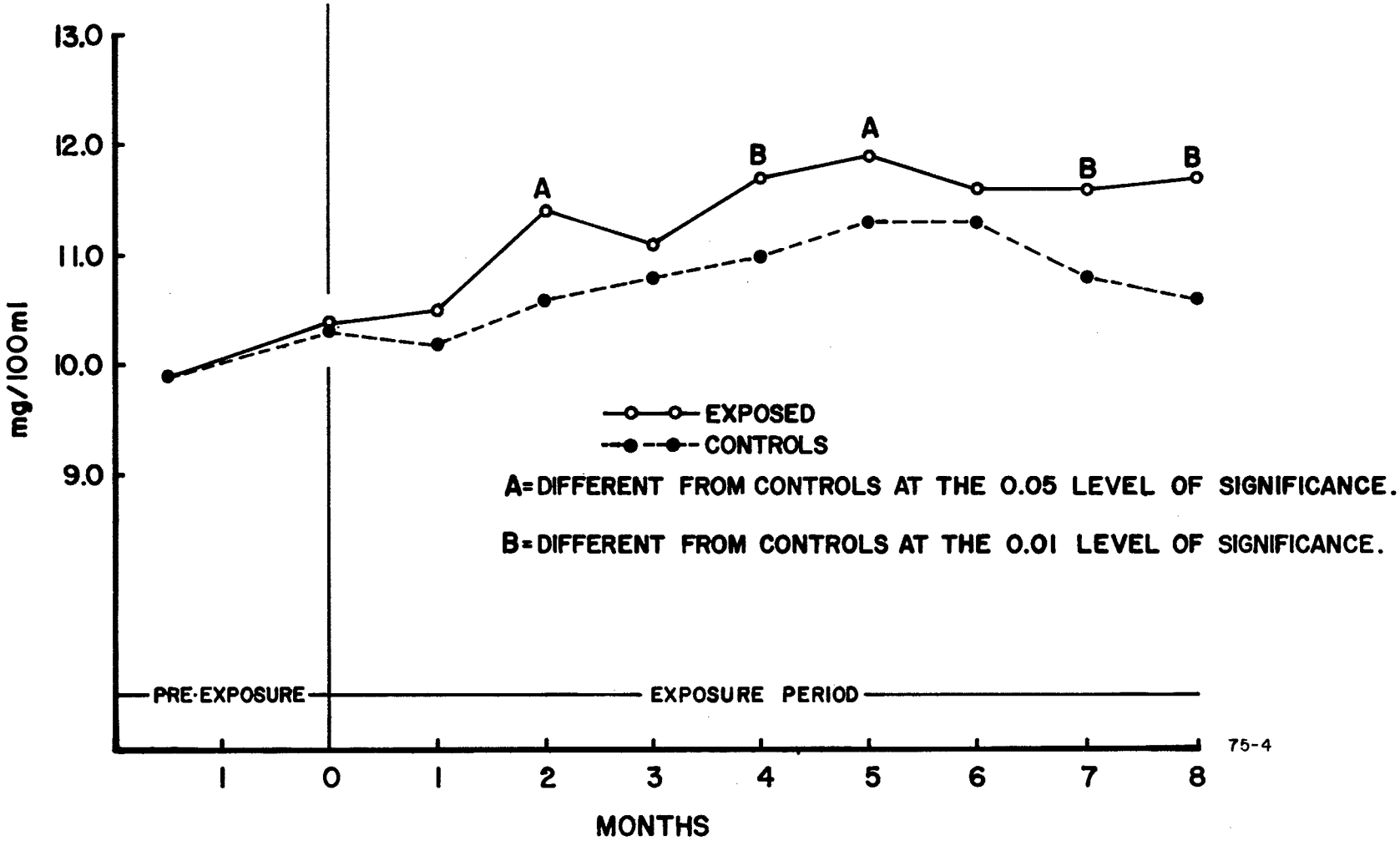


Figure 11. Mean calcium values in monkeys exposed to 10 mg/m^3 coal tar aerosol (N = 14).

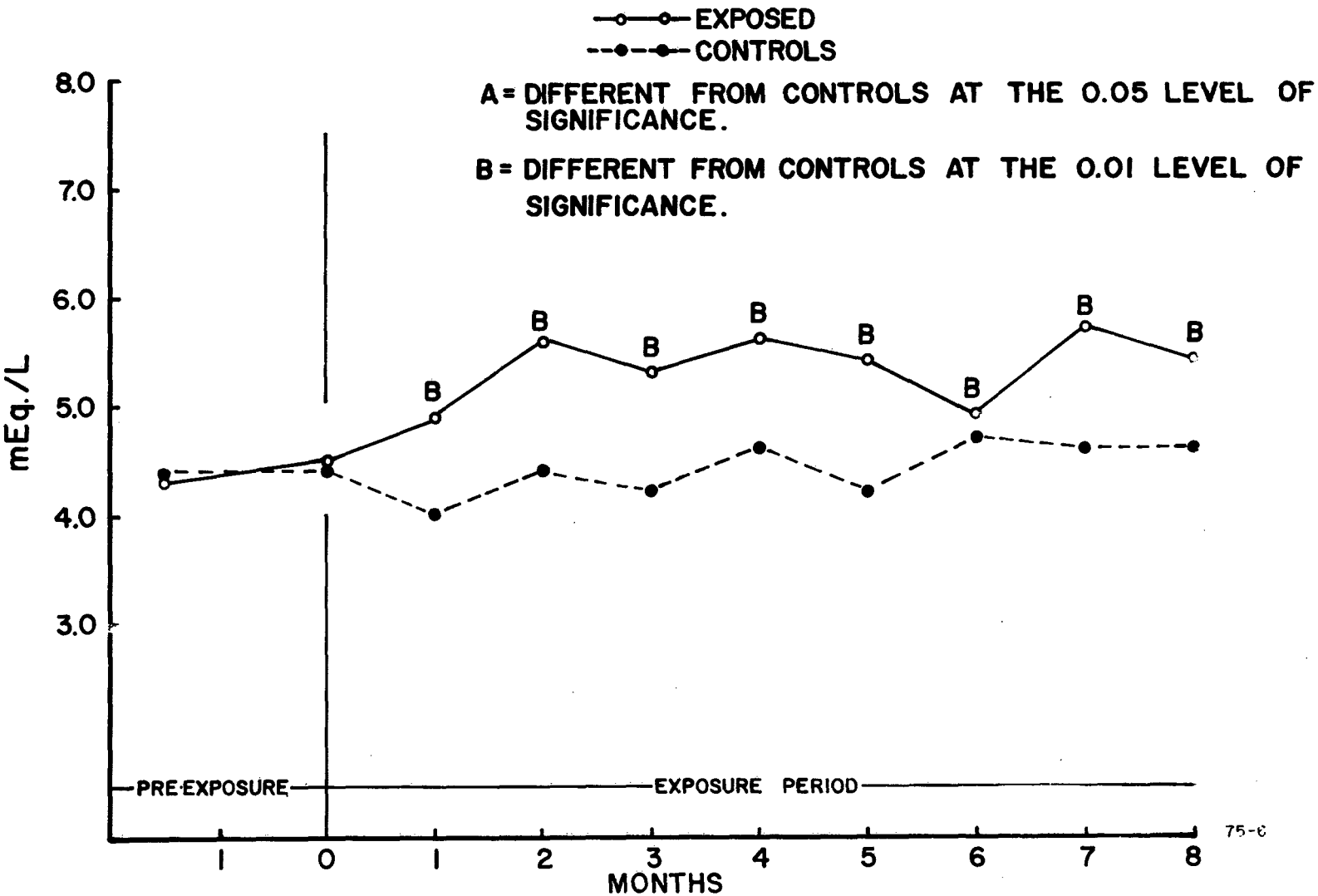


Figure 12. Mean potassium values in monkeys exposed to 10 mg/m^3 coal tar aerosol (N = 14).

Because potassium aids in the regulation of osmotic pressure and acid base balance, blood samples taken from five monkeys showing high values and five controls were examined for these effects. The osmotic erythrocyte fragility values of the exposed monkeys did not differ significantly from the control values. Nor were any differences noted when comparing the pH, CO_2 or O_2 of the exposed monkeys with controls. Blood samples taken from these monkeys during routine blood sampling show no abnormal hemolysis which could lead to high serum potassium determinations. It has not been determined what is causing the high glucose or potassium values. The regulation of potassium in the red blood cells is dependent on glucose metabolism and substances which interfere with this may affect the potassium balance.

A summary of the fluorescence values found in the serially sacrificed CF-1 mice is shown in Table 38. The values show that lung deposition of coal tar continues with time of exposure while hide fluorescence peaks after the first week of exposure. An equilibrium appears to have been established between the amount of coal tar being deposited on the fur and the amount being removed by grooming.

TABLE 38. SUMMARY OF FLUORESCENCE VALUES FOUND IN LUNG AND HIDE OF MICE INTERMITTENTLY EXPOSED TO 10 MG/M^3 COAL TAR AEROSOL

Tissue	Days of Exposure							
	1	7	20	60	108	149	185	233
Lung (μg/g)	6.4	18.5	173	180	210	236	332	315
Hide (μg/cm^2)	1.4	3.2	4.9	3.8	3.7	2.7	3.5	3.2

*Values are test animal values minus control animal values.

The amount of fluorescent compounds found in the lungs of these mice is approximately one-half the amount found in the lungs of the mice exposed to similar doses on CT (concentration/time) basis during continuous exposures to the coal tar aerosol, indicating that some clearance from the lung does occur. This would also indicate that a continuous exposure is a much more severe insult to the lungs of the animals than an intermittent exposure for an equivalent time period. The gradual increase in lung burden of inhaled coal tar aerosol does indicate, however, that a carcinogenic challenge is presented to the pulmonary tissue of these animals during intermittent exposure.

No skin tumors were found in any of the exposed animals during the first eight months of exposure. Only one tumor was found, that on

a CF-1 control mouse after 6 months on the study. The mice are examined biweekly while the other animals are examined monthly during the routine weighing procedure.

During daily animal care periods, all remaining food is discarded and replaced with a fresh supply. The automatic watering system is also checked daily during routine maintenance procedures.

After the initiation of the study, the chambers remained closed. All maintenance activities necessitate entering via the airlock. All technicians entering the chambers are required to wear protective clothing and an air supply respirator. The protective clothing consists of disposable coveralls, footgear, hoods and gloves.

Termination of the various species for this study is as follows:

8 January 1976 - Monkeys, rats and CF-1 mice

8 February 1976 - JAX mice

8 March 1976 - rabbits.

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall mission of the laboratory. Included herein are special projects in analytical chemistry, training programs and engineering modifications to the physical research facilities.

Analytical Chemistry Programs

During the past year, the chemistry department of the THRU has continued to exercise its function of developing and overseeing continuous procedures for the analysis of contaminants being tested in the toxicology program. In addition to this primary responsibility, efforts have been directed towards estimation of the concentration of contaminants or metabolic products of contaminants in the blood of experimental animals. In cases where the chemical and physical properties of the contaminant were such as to require nonroutine methods of introduction, the chemistry department has taken on the task of designing, testing and operating the contaminant introduction procedures.

1,1-Dimethylhydrazine in Laboratory Air, Chamber Exhaust and Cooling Water

Because of the possible carcinogenic properties of 1,1-dimethylhydrazine (UDMH), it was necessary to assure ourselves that laboratory air external to the exposure chambers was not contaminated by the chemical during the 6-month exposures presently being performed. Therefore, the most sensitive modification of the chamber analysis technique, reduction of iodine, was used to check laboratory air in the exposure area. In addition, the air being exhausted from the chambers comes into intimate contact with water being continuously circulated through the vacuum pumps. After passing through the pumps, the air is exhausted into the atmosphere at the roof of the laboratory. In order to assess the quantities of UDMH released into the environment, concentrations in both effluent pump water and stack air were measured using variations of the chamber analysis method. Table 39 lists the data obtained.

The fact that no UDMH was found in laboratory air was not unexpected since the Thomas Domes are operated at a slight negative pressure so that any leaks are into the chamber, and all UDMH introduction systems are in hoods connected to the dome vacuum pumps. Analyses made before and after introduction of UDMH into the domes showed that no iodine reducing compounds are transferred to the pump water when UDMH is absent, and

TABLE 39. CONCENTRATIONS OF UDMH IN LABORATORY AIR, EFFLUENT PUMP WATER AND STACK EXHAUST AIR DURING EXPOSURE PERIODS

Sampling Area	Concentration
External Laboratory	< 0.01 ppm*
Effluent Pump Water	
5.0 ppm UDMH Chambers	12 mg/liter**
0.5 + 0.005 ppm UDMH Chambers	2 mg/liter**
Stack Exhaust Air	
5.0 ppm UDMH Chambers	0.075 ppm
0.5 + 0.05 ppm UDMH Chambers	0.025 ppm

*None found, this is the lower detection limit of the method.
**Water flowing at a rate of approximately 2.6 liter/minute.

that approximately 2 hours are required after termination of UDMH introduction to reduce the level of UDMH in the pump water to zero. A material balance demonstrated that about 72% of UDMH introduced into the chambers is collected in the waste water effluent from the water sealed vacuum air pumps and about 8% is exhausted through the stack. It appears that the other 20% is lost through reaction in the exposure chambers and exhaust flow lines.

Physiological Fluid "Fingerprint" Chromatography

With the acquisition by the THRU of a Waters liquid chromatograph and a Hewlett-Packard dual column programmable gas chromatograph, it became possible to analyze volatile substituents of physiological fluids by gas chromatography and nonvolatile ones by liquid chromatography. A program was instituted with the aim of optimizing sample treatment and chromatographic parameters to obtain "fingerprints" of these physiological fluids, e.g. urine and whole blood, serum or plasma. A fingerprint of these fluids consists of a chromatogram with the number of peaks maximized so that small changes caused by exposure to toxic materials can be detected. The fingerprint can be used in 2 ways in the investigation of toxic effects:

1. It can identify the compounds whose concentration is affected and thus lead to some knowledge of the metabolic effects of the toxicant.

2. It can be used to compare the effects of the toxicant in different species and to identify exposed individuals where some uncertainty concerning exposure exists.

Liquid Chromatography

Experiments were performed to separate components of blood plasma and serum on a number of different columns. A Bondapak C_{18}/Corasil reverse phase column allowed most of the components to pass through unseparated, after which only a few peaks were detected. An attempt was made to perform a separation on a Corasil I (silica) column, but this required sample extraction into a nonpolar-solvent since a large amount of polar material, in this case water, would deactivate the column packing. Extraction proved incapable of removing enough material from plasma or serum to be seen by the detectors. A Bondapak Ax/Corasil anion exchange column provided some separation, but reproducibility is poor. Thus far, experiments with the liquid chromatography have served to define some of the areas where careful technique must be applied in order to obtain precise results. For instance, it was found that reconditioning ion exchange columns after each separation must be performed using a gradient almost exactly the reverse of that used in the separation. Great care must be taken when injecting the sample not to plug up the injector or slow sample bleeding into the column will ensue leading to peak tailing.

The major problem thus encountered in liquid chromatography is that the refractive index detector which can sense almost all components in a mixture, lacks sensitivity, and the ultraviolet detector which has satisfactory sensitivity can sense only a relatively small number of blood serum or plasma components.

Gas Chromatography

In the development of the gas chromatographic fingerprint the first problem investigated was sample preparation. The best procedure developed for urine is the acidification of 10 ml of sample in a distillation flask with 0.5 ml of concentrated sulfuric acid followed by distillation. The first drop condensed in the receiver is injected into the flame ionization detector gas chromatograph with dual columns 1/8 inch in diameter and 5 feet in length packed with Chromosorb 101 at a helium carrier gas flow rate of 15 ml/min, programmed from 50 C - 200 C at a rate of 6C/min. This technique yields a chromatogram of 20 peaks whereas only 10 are obtained from a neutral or alkaline urine. In order to obtain a distillate from plasma which is satisfactory for gas chromatography, sodium tungstate is added to the acidified sample before distillation.

Figures 13 and 14 are gas chromatograms obtained from normal rat urine and plasma. Identifications of the peaks were made by Dr.

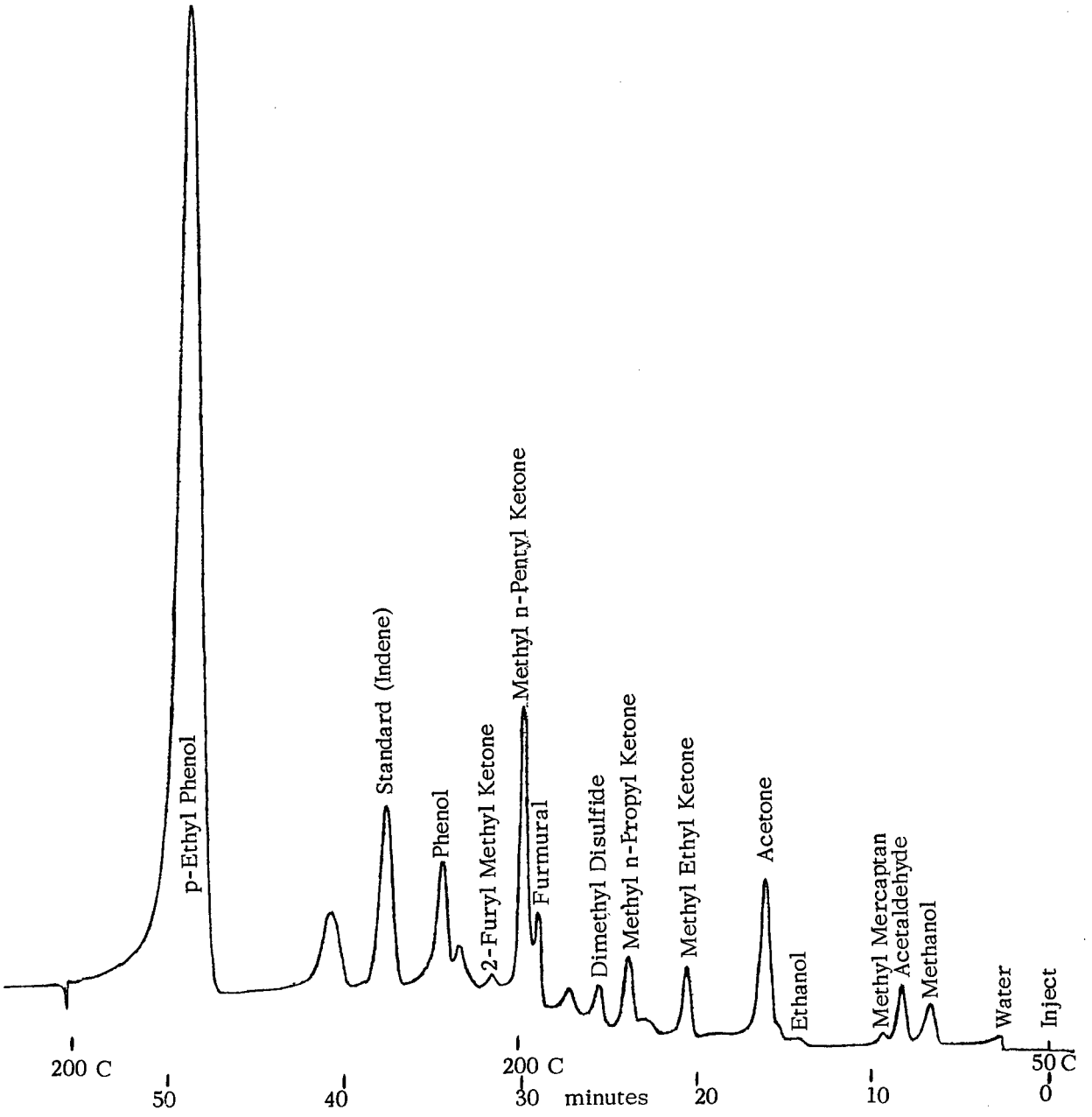


Figure 13. Gas chromatogram of rat urine volatiles.

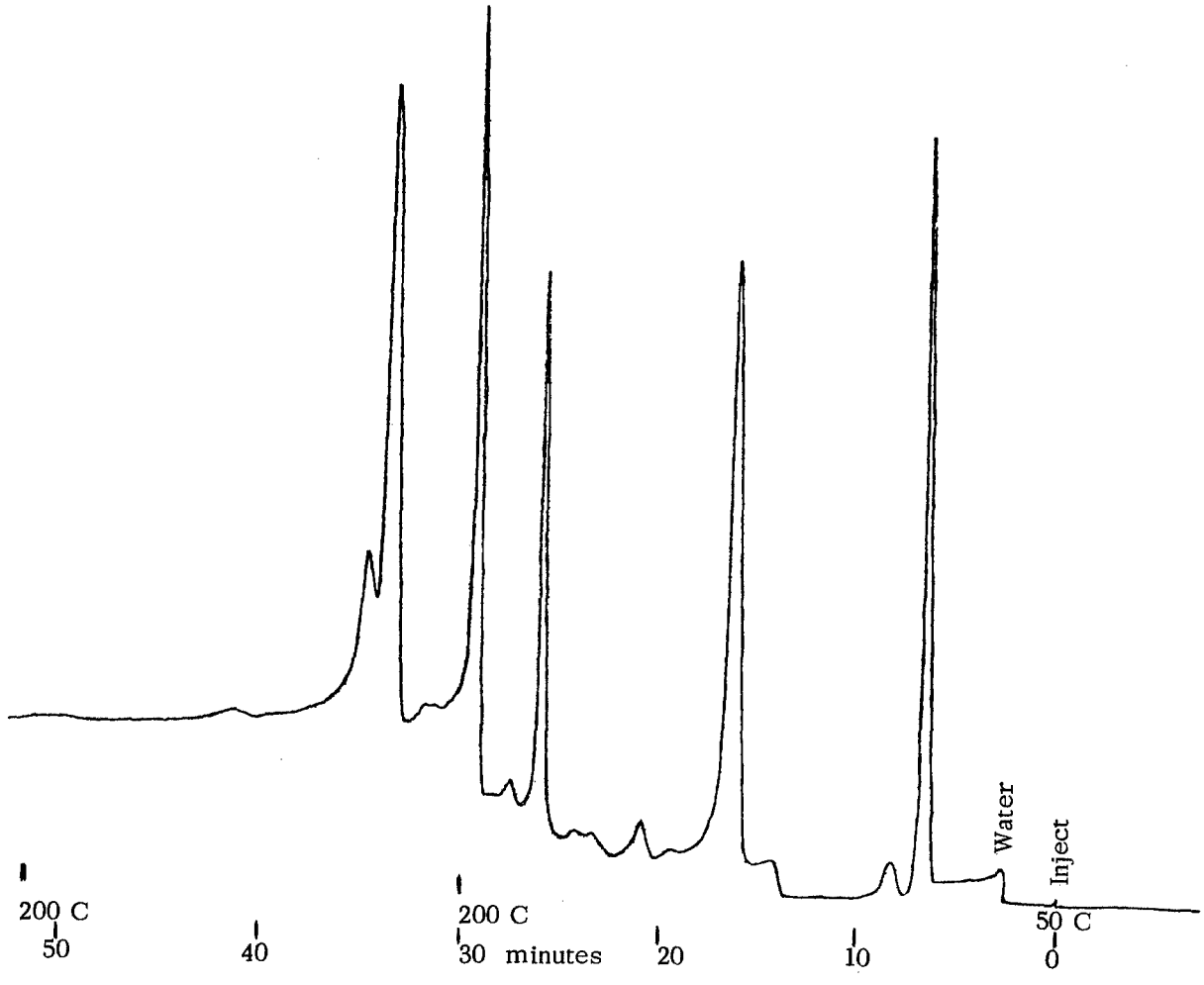


Figure 14. Gas chromatogram of rat blood plasma volatiles.

Kleineberg and Mr. Geiger of 6570 AMRL/THE using gas chromatography mass spectrometry. Work done thus far indicates that chromatograms obtained using urine from the same group of rats are fairly reproducible over long sampling periods, e.g. 1 or 2 weeks. Preliminary experiments indicate that 24 hour fasting and intoxication with carbon tetrachloride or monomethylhydrazine produce characteristic changes in the chromatogram obtained from urine. Since animals may become anorexic after intoxication, some care is required in interpreting changes in urine chromatograms.

Osmotic Fragility of Red Blood Cells

Red cells suspended in hypotonic solutions of sodium chloride (NaCl) take up water, swell and, if the salt concentration is sufficiently dilute, eventually burst. Normal red blood cells of any species exhibit a characteristic distribution of percent hemolysis over a range of salt concentrations. Since some of the compounds of great toxicological interest to the Air Force such as monomethylhydrazine and 1,1-dimethylhydrazine, which induce Heinz body hemolytic anemia, affect the distribution of hemolysis of red blood cells sampled from exposed animals, particularly dogs, osmotic fragility is an important and frequently performed determination. The standard clinical laboratory procedure is a manual method requiring a great deal of sample manipulation and taking

considerable time (Davidsohn and Henry, 1969). In order to handle the numbers of samples required in recent studies on the various hydrazines, MMH, UDMH and hydrazine itself, it was necessary to reduce the time expended in the determination. This was accomplished by automating the spectrophotometric measurement of lysed hemoglobin using a Technicon AutoAnalyzer® sampler and a flow cuvette in the Coleman Autoset® spectrophotometer according to the following procedure:

Equipment

1. Tray for holding 3 ml sample vials
2. Volumetric glassware (including 50 μl micropipette)
3. Clay Adams Safeguard Table Top Centrifuge (12 samples)
4. Technicon AutoAnalyzer® Sampler
5. Buchler Polystaltic® Pump
6. Coleman Autoset® with flow-through cuvette
7. Varian millivolt recorder.

Reagents

1. Stock solution (pH 7.4, osmolal equivalent of 10% NaCl)

NaCl	180.00 g
Na_2HPO_4	27.31
$NaH_2PO_4 \cdot 2H_2O$	4.86

Dissolve in water and dilute to 2 liters.

2. Dilute stock solution with distilled water to obtain the osmolal equivalents of the following percentage NaCl solutions,

0.85	0.55	0.35
0.70	0.50	0.30
0.65	0.45	0.20
0.60	0.40	0.10

3. Sample and control blood samples (with anticoagulant) should be obtained and treated at the same time.

Procedure

1. 2.0 ml of each salt solution is added to the 3 ml vials in a row.
2. 50 μl of blood is added to each tube and stirred by air blown through the sampling pipette.
3. The tubes are incubated at room temperature for 20 minutes then transferred to the centrifuge for clarification at 2000 rpm for 5 minutes.
4. Following centrifugation, the tubes are placed in order of decreasing salt concentration on the AutoAnalyzer® with four water blanks separating samples.
5. The pump is set to remove 1.7 ml of supernatant with a small air bubble to separate dilutions.

6. The sample passes through the pump, a minimal amount of transmission line and the flow cuvette, then to waste.
7. Absorbance at 540 nm is used to calculate % lysis.

100% = Absorbance at 0.10% saline

0% = Absorbance at 0.85% saline.

With the method described above and the sampler operating at one/minute, at least three blood samples (12 salt dilutions each) can be examined per hour. Using this procedure, the THRU chemistry department was able to accomplish all of the osmotic fragility determinations scheduled during various toxicological investigations. There were 2 questions concerning the treatment of sampled blood. The first concerned possibly different effects on osmotic fragility of the use of EDTA or heparin as anticoagulants. The second had to do with the consequences of refrigerated storage of blood after sampling. In order to ascertain what, if any, these effects might be, blood was drawn from a monkey and divided into 2 samples, one using EDTA and the other heparin as anticoagulants. Osmotic fragility was determined 1, 2, 3, 4, 5, 6 and 30 hours after sampling. Figure 15 illustrates the results obtained using percent hemolysis at 0.40%, 0.45% and 0.50% equivalent NaCl solutions which cover the range from low to high hemolytic effects. There appear to be differences caused by the use of different anticoagulants. While

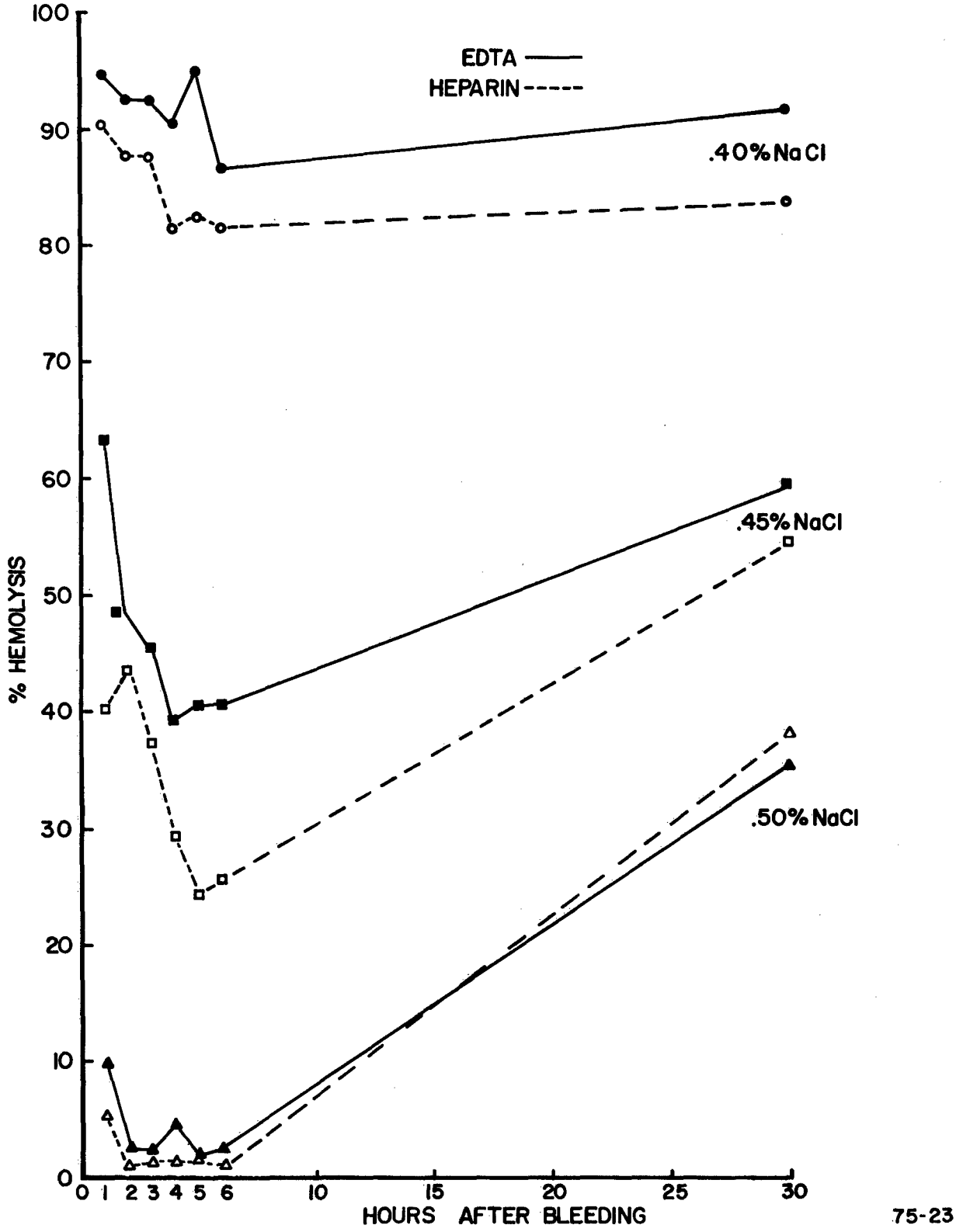


Figure 15. Comparison of osmotic fragility of RBC's in EDTA or heparin.

the differences are not great, erroneous conclusions could be derived if the anticoagulant was changed during a study. Overnight storage of blood in EDTA or heparin produced the same results, greatly increased hemolysis with the greatest changes occurring in the region of least hemolysis. The samples tested at 2 to 6 hours after bleeding generally indicated only slight differences at the 0.40, 0.45 and 0.50% NaCl solution levels. It has, therefore, become standard practice to test blood approximately 2-3 hours after being drawn.

Modification of UDMH Analysis in Animal Exposure Chambers

The UDMH analysis was originally set up so that one analyzer would serve two exposure chambers which were nominally operated at the same concentration. Sampling was accomplished by timed solenoid valves which alternately switched chamber air samples through one absorbing tower before analysis by the colorimeter. The system required a minimum of one-half hour sampling time per chamber to obtain meaningful results. This length of analysis time was necessary because equilibrium in the dome sampling lines and absorber was upset during the time that the sample was not being pumped through the absorber and required a significant time to be re-established after swtiching the sample solenoid. Since the exposures were only six hours in length per day, control of the domes could be improved by decreasing the required sample time.

To shorten the sampling and analysis time, a system was designed whereby air samples were drawn from each dome continuously and passed through separate absorbing towers. An electrically operated gas chromatograph (GC) injector valve was adapted to alternately switch the iodine absorber solutions from each of the two towers into the colorimeter.

Figure 16 depicts the operation of the GC injector valve. By using continuous dome air sample flow and two absorber towers, the domes could be sampled every ten minutes and produce eight minutes of useful information per sample. Although the GC valve was satisfactory for the purpose of demonstrating the feasibility of the proposed sampling system, it could not be used routinely since the metal surfaces were corroded by the absorber solution. The system requires inert plastic solenoid operated valves. Sources for these valves are being investigated and they will be integrated into all dome analysis systems.

Properties, Generation and Measurement of Aerosols

For a number of years, the importance of aerosols in inhalation toxicology has been recognized. Aerosols can have specific toxic effects of their own as in the cases of silica, asbestos or beryllium oxide. They may potentiate the toxicity of gases by adsorbing or dissolving them and permitting penetration into the deep lung. Conceivably they may also moderate the toxic effects of some gases after adsorption or dissolution by reaction or by catalyzing reaction or decomposition of the toxic gas. Because of the toxicological

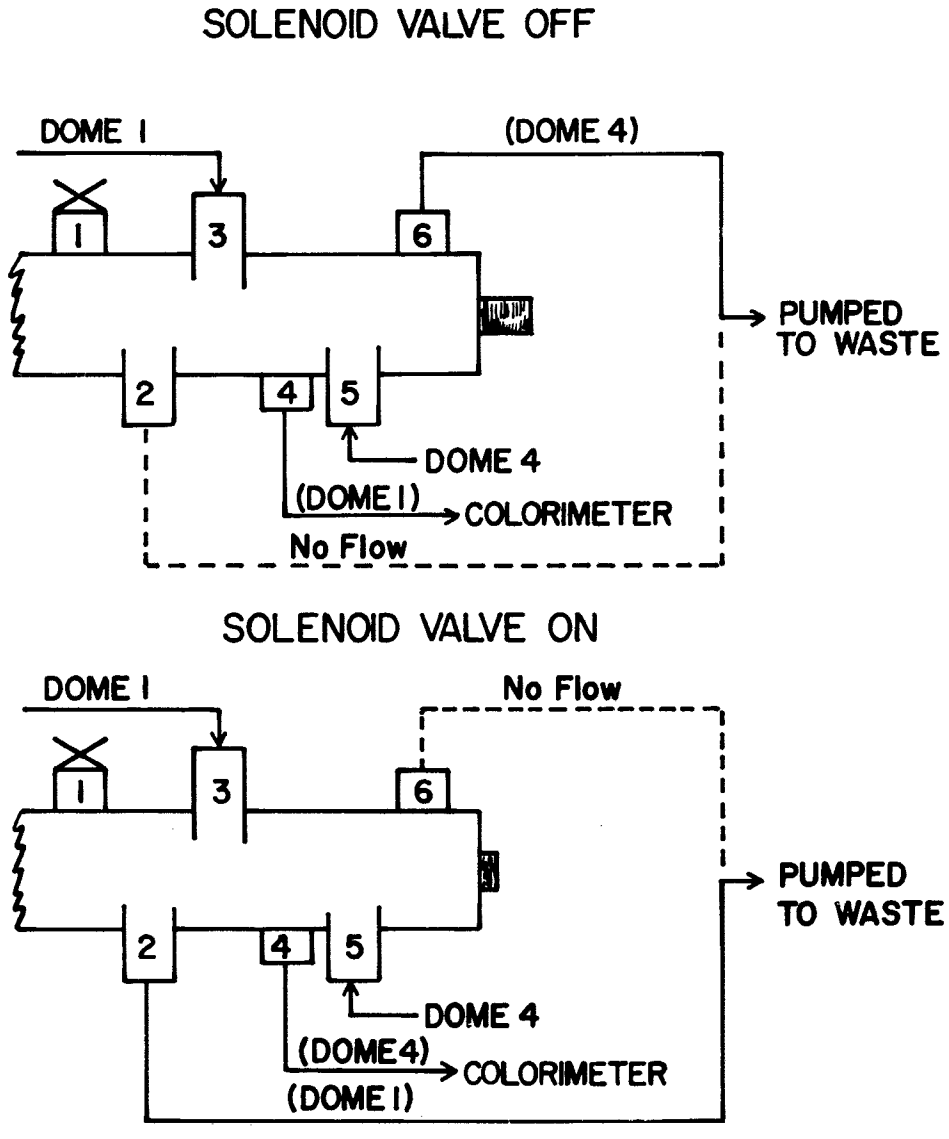


Figure 16. Modified chamber atmosphere sampling system.

importance of aerosols, the THRU has acquired a considerable amount of instrumentation for the characterization of aerosols and has initiated a comprehensive investigation into the physical properties of aerosols. As a first step in this investigation, a review was presented at one of the monthly THRU seminars. This review is included in the annual report because of its lucid analysis of the problems and procedures associated with the investigation of aerosol toxicology.

I. Coagulation

Coagulation is the term used to describe the process of adhesion or fusion of aerosol particles upon contact with each other.

Thermal coagulation describes the process when Brownian motion is the only factor effecting particle movement leading to contact. The number N_{Th} of contacts resulting in coagulation is the product of the total particle concentration C_o per cubic centimeter and the thermal coagulation constant K_o:

$$N_{Th} = K_o C_o \tag{1}$$

The constant K_o is calculated from diffusion theory. The change in concentration of particles due to thermal coagulation is calculated from equation 2:

$$C = \frac{C_o}{1 + \frac{tC_oK_o}{2}} \tag{2}$$

Attractive forces between particles can also cause coagulation. Van der Waals forces between particles lead to particle collisions. The molecular coagulation constant Z has a value of ~1.5 increasing K_o by a factor of Z in equation 2 which becomes equation 3.

$$C = \frac{C_o}{1 + \frac{tC_oK_oZ}{2}} \tag{3}$$

Brownian motion and Van der Waals forces affect the size distribution of all aerosols making monodisperse aerosols difficult to obtain.

Charged particles increase coagulation. The charge coagulation constant Z_c added to equation 3 gives 4.

$$C = \frac{C_o}{1 + \frac{tC_oK_oZZ_c}{2}} \tag{4}$$

Electrical and magnetic dipoles increase the coagulation rate. The dipole coagulation constant Z_d is equal to the ratio of the distance between dipoles (M) and the distance between the particle centers (d). Equation 4 becomes 5.

$$C = \frac{C_o}{1 + \frac{tC_oK_oZZ_cM}{2d}} \tag{5}$$

The application of external forces cause particle coagulation. Direct coagulation is the result of electrical or magnetic fields. K_o is increased by the factor $\overline{Z}$ which equals:

$$\overline{Z} = \frac{(\text{field strength})^2\, r^3}{12kT} \tag{6}$$

Kinematic coagulation is the result of gravitational or centrifugal forces. A large particle with radius (R) moving through an aerosol with radius (r) will collect the smaller particles at the rate,

$$N/sec = \left(\frac{R + r}{R}\right)^2 \pi R^2 V_S \tag{7}$$

where V_S is the sedimentation rate of the large particle. Ultrasonic vibrations will cause aerosols to coagulate to a small extent.

Aerosols moving in laminar or turbulent air flows will tend to coagulate. The number of collisons expected in a laminar flow system will be,

$$N_L = \frac{32}{3} C_o G r^3 \tag{8}$$

The term G is the velocity gradient perpendicular to the laminar flow. In the case of turbulent flow,

$$N_T = 25\left(\frac{E}{n}\right) r^3 C_o \tag{9}$$

where E is the energy loss and n is the air viscosity.

II. Electrical Charge

Most aerosols possess electrostatic surface charges. The physical properties of the particle help determine the maximum limit of the charge. Electron, ion and Rayleigh are the limits associated with aerosol particles. The electron and ion limiting charges are related to the particle diameter and the surface field intensity produced by the charge. Rayleigh limits are

a function of particle surface tension and drop diameter, and are associated with liquid aerosols.

Bipolar ions, from an external source, will place a change on a particle during collisions with the particle. Small particles $\leq 0.05\,\mu$ are not usually charged as indicated by a total particle to uncharged particle ratio of 1. As particle size increases, this ratio also increases. $1.0\,\mu$ particles have a ratio of 7.5 when exposed to bipolar ions indicating an increase of charged particles.

Unipolar ions will charge aerosol particles. When an external electrical field is applied, the motion of the ions becomes ordered and directed with the field. Aerosols moving perpendicular to the field will collide with the ions and become charged.

Particles charged by unipolar ions will also move in the direction of the applied uniform field. This electrical mobility of the charged particle is defined as follows:

$$Z_p = \frac{n_p e}{3\pi n D_p C} \qquad (10)$$

where $n_p e$ is the particle charge, n is the air viscosity, D_p is the particle diameter and C is a constant. Figure 17 shows particle mobility versus various particle sizes with unit charge.

III. Generation

Aerosols are formed in two ways; dispersion and condensation.

Dispersion: Atomization of a liquid is the easiest technique to use. Liquids with low vapor pressures and suspensions of solids in liquids can be atomized. The main types of atomizers are the air-blast type, the hydrodynamic type and the rotating disc.

The air blast type uses air moving at high velocity to break up a liquid stream coming from a nozzle. Aerosols generated this way are polydisperse, having a wide particle size distribution with a mean particle diameter of 100-500 μ.

The hydrodynamic generator forces the liquid through a nozzle at very high pressures. The size of the droplets formed is dependent on the physical properties of the liquid, e.g. viscosity and surface tension.

The large particle sizes of these generators may be reduced to the 1 μ and below range by impaction on baffles or reservoir walls. Impaction leads to greater polydispersity. Vertical columns with baffle plates to size segregate the particles are used to obtain monodisperse aerosols.

The third type of atomizer is the rotating disc. The liquid to be atomized is dropped to the center of a disc rotating between 50 and 5000 r.p.s. The liquid is centrifuged from the edge of the disc as small particles. This atomizer produces monodisperse aerosols in the size range of 1-100 μ. The particle size is controlled by the liquid delivery rate and disc spinning rate. The spinning disc atomizer is also capable of producing high concentrations of aerosols.

Dispersion of solids is accomplished in one of two ways. The first has been mentioned, atomization of solids in liquid suspension. The second is to grind the solid to the required size and then disperse into an air stream. An advantage here is that particle size may be chosen prior to introduction. A Wright dust feeder scrapes the particles from a packed bed. The particles are impacted on a plate to break up aggregates before dispersal into the air stream. When the material has low density and small particle size, it may be aspirated from a highly stirred bed into a second chamber, where the particles are impacted on the walls and stirred. Particles are then ejected into the main air stream. The technique is fluidization.

Condensation: This method produces aerosols of the highest monodispersity. The heat exchange generator operates on the principle of super saturating with the material being aerosolized and subsequent condensation on nuclei.

In Figure 18 the liquid is atomized into the segregator where the large particles are removed. In the vaporizer, the droplets are completely evaporated. Condensation takes place in the cooler on nuclei which rise from nonvolatile impurities in the liquid. The length of the cooler determines the size of the aerosol generated. Dilution of the stream leaving the cooler will decrease coagulation to maintain monodispersity. This generator is valuable for liquids and solids that will vaporize without decomposition. The generator produces aerosols with a mean particle diameter of 0.1 to 1 μ.

The mixer type generator in Figure 19 uses a stream of air passing through a heated bed of silica gel impregnated with the liquid. A jet of vaporized liquid passes into a cone where it is mixed with a jet of cold air flowing four times the vaporizer. Condensation occurs on small particles of silica gel. The mixer produces aerosols of 1 μ and less mean particle diameter.

Chemical reactions can form aerosols by condensation. An aqueous condensation aerosol forms when gaseous ammonia and gaseous HCl react. The ammonium chloride acts as condensation nuclei for water vapor.

Combustion products form condensation aerosols with water vapor.

IV. Collection

Sampling of aerosols is divided into two cagegories, bulk sampling and size separation and classification during sampling. Whichever category is chosen, acquiring a representative sample from a moving stream requires isokinetic sampling. Figure 20 illustrates this principle.

Isokinetic: sample line velocity equals particle stream velocity. Sample line is parallel. No particle size differentiation occurs.

Anisokinetic: Sample line velocity less than particle stream velocity. Small particles will be carried past the sample probe and sample will have an excess of large particles. If the sample line velocity is greater than particle stream velocity, an excess of small particles will enter the port and the sample will be deficient in larger particles.

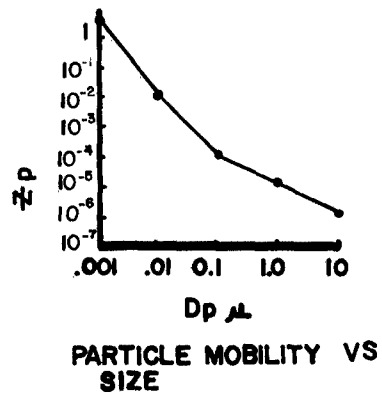


Fig. 17

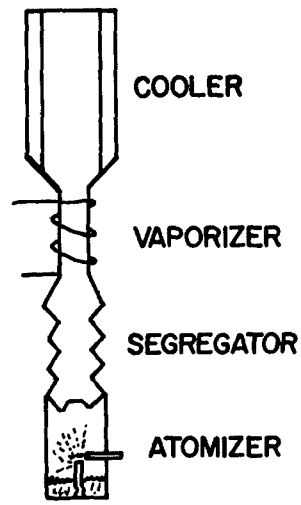


Fig. 18

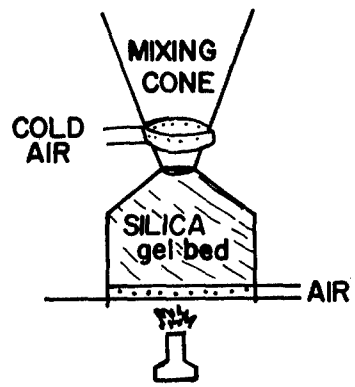


Fig. 19

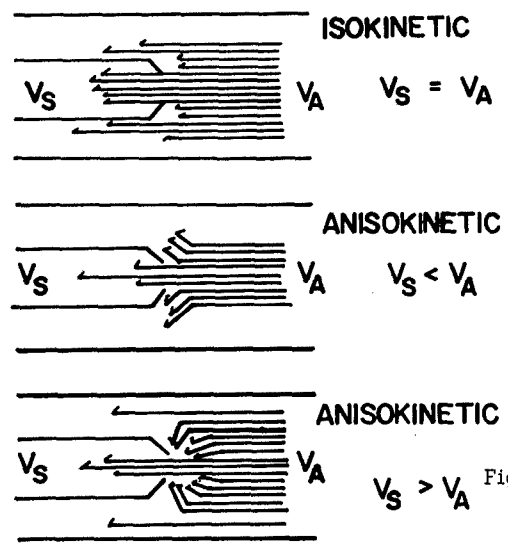


Fig. 20

Figure 17. Effect of particle diameter on mobility.
Figure 18. Vaporizing apparatus for production of aerosols by condensation.
Figure 19. Heated bed apparatus for production of aerosols.
Figure 20. Air flow patterns created by different sampling rates.

When sampling aerosols with particles greater than 5 μ, isokinetic conditions are mandatory. Aerosols with the largest particle 1 μ are not greatly effected by deviations from isokinetic sampling.

Bulk Sampling: Settling chambers use gravity and low flow rates through a horizontal chamber. Samples of aerosols down to 5 μ are collected in trays or on slides.

Impingers collect aerosols by impacting a jet on a flat surface ~ 5 mm below the jet nozzle. The impinger may or may not contain a liquid medium, depending on the method of size analysis to be used. Impingers are efficient down to 0.5 μ size particles.

In passing an aerosol stream over a wire heated to 100 C and between cool walls, thermal precipitation will occur. A thermal gradient results causing 20 μ or smaller particles to migrate to the cool walls.

Electrostatic precipitation results when an aerosol is charged by a high voltage unipolar corona discharge. An electric field drives the charged particles to a collection plate. This method is 100% efficient for 5 μ and smaller particles.

Filters of soluble sugar or salt, membranes of cellulose ester gel, paper and fibrous materials have been commonly used to collect aerosols.

Sample classifying: The most common classifier is the cascade impactor. The aerosol is passed through a series of jets of decreasing diameter. The total air volume drawn through the sampler is constant but the

velocity through each smaller jet increases. The efficiency for collecting smaller particles increases as the velocity increases.

V. Analysis

The collection methods mentioned above are intended for aerosol analysis and sizing by subsequent methodology. Sizing and distribution analysis are commonly done by optical or electron microscopy. Determination of aerosol composition is accomplished by x-ray analysis or mass spectrometry. Some of the new instrumentation allows the automatic counting and sizing of aerosols. In our laboratory, we have several instruments useful in characterizing aerosols.

Sinclair-Phoenix Photometer: Uses near forward light scattering to measure aerosol concentration. Filter samples can be collected for particle size analysis. Aerosol concentrations in the range 10^{-5} μg/L to 100 μg/L can be measured.

Royco Particle Counter: Uses forward light scattering to count aerosol particles. The intensity of light striking a photomultiplier gives rise to a current which is proportional to particle size. Sampling is done in such a way that light scattered from an individual particle is seen as a pulse by the tube. The individual current pulses arising are stored in a memory. At the end of a specific sampling cycle, the counts in the memory channels are recorded. The instrument is capable of counting 100,000

particles/min from a 1 CFM sample, and is equipped with channels for 5 size ranges:

<0.7μ

0.7 - 1.4μ

1.4 - 3.0μ

3.0 - 5.0μ

>5.0μ

Thermo Systems Aerosol Neutralizer: Removes charges from aerosol particles using a Krypton 85 source. The uncharged aerosol particles are then available for analysis by the following instrumentation.

Thermo Systems Electrostatic Precipitator: Uses a unipolar corona discharge to place a charge on neutral particles. The charged particles are precipitated onto electron microscope grids by a uniform electrical field in a second chamber.

Thermo Systems Aerosol Size Analyzer: Uses the mobility properties of charged particles to size differentiate over the range 0.003 to 1.0μ. The instrument consists of three sections, a corona charger, a precipitator and a current sensor. Five L/min sample containing neutral particles is exposed to a unipolar corona discharge of positive ions. The charged particles travel to the precipitator where a uniform electrical field drives the particles to a collecting rod. Particles with high electrical mobility are

collected while the larger particles migrate to the current sensor. Varying the field strength stepwise gives cut-off sizes that will not pass the precipitator. The current sensor collects the charged particles on an absolute filter where an electrometer reads the charge density and records the current. Data are tabulated in Table 40. D_p is the cut-off size giving current I. D_{pi} is the mean particle diameter between cut-off sizes. $[\Delta N/\Delta I]$ is a calculator constant. ΔI is the current between successive steps. The product $[\Delta N/\Delta I]\Delta I$ gives the concentration of particles per cm^3.

VI. Treatment of Particle Size Data

Graphic analysis of particle size data is a common approach (shown in Figure 21. A normal shaped curve (Figure 21A) will give the mean diameter as the value $d\mu = \Sigma d\mu_i/N$ which will divide the area under the curve into 2 equal parts. The distribution of sizes is represented by the first standard deviation σ_g as a measure of the width of the curve. Sixty-eight percent of all observed particle sizes will be within σ_g.

Most particle size data will not give a normal shaped distribution curve, rather a skewed one (as Figure 21B). The mean $d\mu$ may be weighed by fewer large (or small) particles of $d\mu$. The median particle diameter $d\mu$ will be a more representative value in this case. The median value will divide the area under the skewed curve into equal parts.

TABLE 40. VARIATION OF ELECTRICAL MOBILITY OF AEROSOLS WITH PARTICLE SIZE

Position	Collector Voltage (V)	D_p - μm	D_{pi} - μm	$\Delta N/\Delta I$ - X 10^{12} amps
1	20	.0032		
			.0056	4.659 X 10^6
3	196	.0100		
			.0178	6.051 X 10^5
5	1220	.0316		
			.0562	6.365 X 10^4
7	3515	.100		
			.178	1.849 X 10^4
9	7152	.316		
			.562	6.051 X 10^3
11	9647	1.00		

When particle size data are plotted as the log $d\mu$ vs. frequency, a normal shaped curve may result, designated as the log-normal curve (see in Figure 21C. The median value $d\mu$ will coincide with this curve's mean value log $d\mu$.

A more useful representation is obtained by using cumulative plots of % of particles by count less than or equal to a given size versus log particle diameter. Here the median value log $d\mu$ will be that for 50% (shown in Figure 21D).

Figure 21E shows that the same cumulative data as Figure 21D on log probits paper will result in a straight line in most cases. The median value log $d\mu$ will still occur at 50% and is called Mg, the number mean diameter. The slope of the line is σ_g and is the size distribution as determined by

$$\frac{\log d\mu_{50\%}}{\log d\mu_{15.89\%}} \quad \text{or} \quad \frac{\log d\mu_{84.13\%}}{\log d\mu_{50\%}}$$

Table 41 shows some of the more common terms used in particle size analysis. Mg' is the mass median particle diameter where one-half of the total mass of the material is represented by particles whose diameters are greater than Mg'.

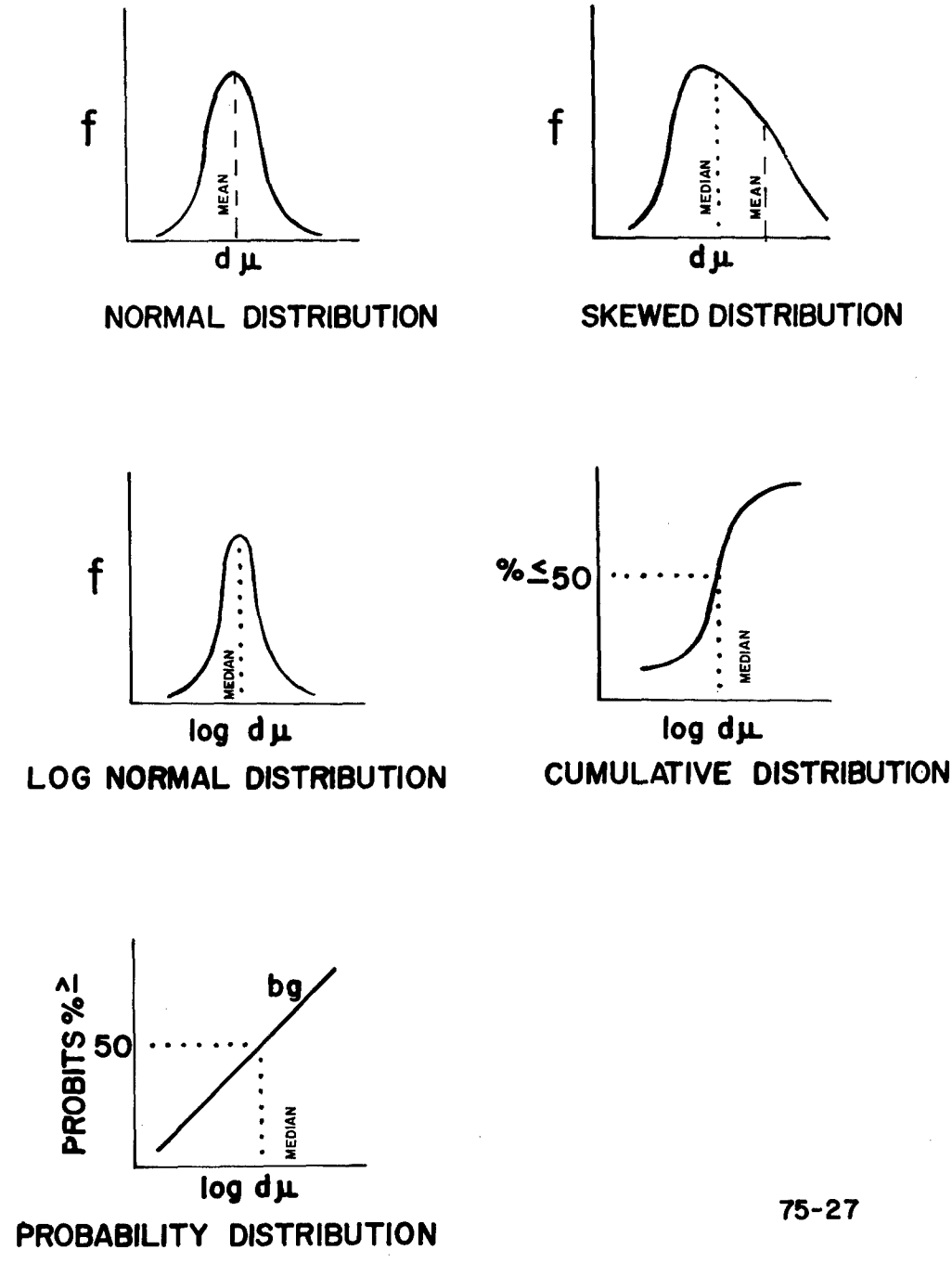


Figure 21. Particle size distribution curves.

TABLE 41. PARTICLE SIZE TERMS AS OBTAINED LOG NORMAL - PROBABILITY DISTRIBUTIONS

Particle Size	Log Value By Count	Log Value by Weight
Geometric Mean Mg	$\log Mg$	$\log Mg' - 6.9078 (\log \sigma_g')$
Arithmetic Mean σ	$\log Mg + 1.1513 (\log \sigma_g)^2$	$\log Mg' - 5.7565 (\log \sigma_g')$
Specific Surface d_s	$\log Mg - 1.1513 (\log \sigma_g)^2$	$\log Mg' - 8.0591 (\log \sigma_g')$
Surface Area Δ	$\log Mg + 2.3026 (\log \sigma_g)^2$	$\log Mg' - 4.6052 (\log \sigma_g')$
Volume D_v	$\log Mg + 3.4539 (\log \sigma_g)^2$	$\log Mg' - 3.4539 (\log \sigma_g')$
Surface Area Per Unit Volume D_v^3/Δ^2	$\log Mg + 5.7565 (\log \sigma_g)^2$	$\log Mg' - 1.1513 (\log \sigma_g')$

Pollutant Gas Interaction

The University of California, Irvine (UCI) has instituted a program for investigation of the interaction of air pollutant gases and aerosols. This program was drawn up because of the considerable evidence (McJilton et al., 1973; Amdur and Underhill, 1968; Frank et al., 1964) that aerosols can potentiate the toxic action of gases or vapors. Laboratory experiments have demonstrated that sulfur dioxide is more easily carried into the deep respiratory areas when accompanied by an aqueous aerosol than when used alone. Recent studies (EPA Document APTD-1466, 1973) have shown that inorganic sulfate aerosols are present in East Coast smog, and that sulfate and nitrate aerosols exist in Los Angeles Basic smog. Therefore, UCI is undertaking a comprehensive investigation into the interactions of ammonium sulfate and ammonium nitrate aerosols with ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide.

Before tests could begin on the effects of combining pollutant gases and aerosols, it was necessary to determine if there are interactions among the gaseous pollutants themselves. The gases chosen for the initial investigation were O_3 and NO_2, and the THRU was given the task of examining their interaction. These experiments were performed to implement research objectives of the University of California.

Initial formulation of the experimental approach was to determine threshold concentrations of individual gases for effect on 3 physiological parameters: wet lung weight and/or lung water, respiratory rate and histopathological changes. While investigations into sensitive techniques of measurement of respiratory rates and histopathological changes were taking place, it was decided to proceed with the study using wet lung weight and lung water increases after 4 hour exposure to the toxic gases as measures of effect. In this approach, the threshold concentration was defined as that causing the smallest significant difference between test and control group means. After measurement of the coefficient of variation of rat lungs in a group, it was calculated that the smallest difference in group means of 20 animals which would have statistical significance was 5%.

The first problem which required solution in the investigation of the effects of low levels of ozone and nitrogen dioxide was analytical. The requirements of a chemical analytical procedure for chamber atmospheres were:

1. Continuous analysis so that the chamber pollutant concentration would be known at all times during the exposure.
2. Rapid response so that any change in chamber concentration would be quickly detected and remedied.

The THRU chemistry department, therefore, adapted standard colorimetric procedures for O_3 and NC_2 to the Technicon AutoAnalyzer® continuous analyzer. The Lyshkow (1965) modification of the Saltzman method was found satisfactory for the analysis of NO_2 alone and when C_3 was present. The standard EPA iodimetric method for O_3 (Federal Register, 1971) was successfully automated and used in the early studies with O_3 as the sole contaminant. However, it could not be employed in the presence of NO_2 which interfered. A Dasibi Ozone Monitor was borrowed from UCI and used in exposures to mixtures. Since this instrument measures UV light absorbed by O_3, no interference is given by NO_2. Experiments performed on pure O_3 mixtures demonstrated that identical results were given by iodimetric and Dasibi procedures.

NO_2 was delivered from calibrated mixtures of 1% NO_2 in N_2 pressurized to about 1000 psi. O_3 was generated using Sander Model III ozonizers. Pure oxygen had to be used as a source rather than air to avoid formation of some NO_2.

Since the edematous effects of acute exposure to deep lung irritants are sometimes delayed, the experiments were designed to take this into consideration with measurements of lung weight made at varying times after exposure until a maximum was reached. We planned to use this sacrifice time routinely in all subsequent exposures. As it turned out, the time required to produce maximum increase in lung weight was different for the mixtures than for the pure compounds and may have varied slightly with concentration of the pure materials. Although rat lungs were weighed immediately after sacrifice and after drying at 100 C for 24 hours to yield measurements of wet and dry lung weights and lung water, the wet lung weights were more precise indicators of edema than lung water. This might have been expected since wet lung is measured with one weighing while lung water requires two. Also, lung water and lung solids increased in approximately the same percentage in exposed animals over controls. This indicates that lung edema fluid produced by exposure to irritating gases contains approximately the same ratio of water to solids as normal rat lungs.

One of the assumptions upon which this study was based was that rats of equal body weights would have equal lung weights, and that the lungs would increase equally in weight after exposure to identical concentrations of irritant gases. However, when 2 groups of rats from different

shipments were given 4 hour exposures to mixtures of 2.0 ppm O_3 and 15 ppm NO_2, these assumptions did not hold. The data are shown in Table 42.

TABLE 42. EFFECT OF EXPOSURE TO A MIXTURE OF O_3 AND NO_2 ON RAT LUNG PARAMETERS

	Wet Wt. Gms.	Dry Wt. Gms.	Lung H_2O Gms.	Body Wt. Gms.	N
Group A					
Controls	1.4890	0.3462	1.1428	253.1	10
Sac. Immed.	1.6762	0.3632	1.2120	253.8	10
Sac. 24 Hrs.	1.6179	0.3969	1.2210	251.5	10
Group B					
Controls	1.0979	0.2649	0.8330	252.3	10
Sac. Immed.	1.3778	0.2977	1.0801	256.4	10
Sac. 24 Hrs.	1.3569	0.3083	1.0486	245.8	10

Comparison of the two control groups clearly shows that Group A had significantly lighter lungs than Group B even though they were the same strain and age and their body weights were almost identical. The increase in lung weight after exposure was greater in the animals with light lungs in both a relative and absolute sense. Although this was a rare occurrence, it illustrates that the variation among groups is probably larger than anticipated. With few exceptions, the mean body weights of the rats used in this study ranged from 240 to 300 grams and the control lung weights from 1.3 to 1.7 grams.

The differences between lung weights of control animals and those exposed for 4 hours to various concentrations of O_3 or NO_2 are listed in Table 43. Table 44 contains the data for mixtures of O_3 and NO_2.

TABLE 43. EFFECT OF A SINGLE 4-HOUR EXPOSURE TO O_3 OR NO_2 ON RAT LUNG WEIGHTS

Gas Conc. ppm	Postexposure Time to Sacrifice, hrs.	N	Lung Weight Difference from Controls	
			Grams	Percent of Control Lung Wt.
O_3				
2.5	0	18	0.0317	2.43
2.5	24	20	0.1784	13.16
1.25	0	5	0	0
1.25	48	5	0	0
1.25	96	5	0	0
1.25	168	5	0.0667	3.66
1.9	0	10	0.0290	2.04
1.9	0	10	0.0068	0.44
1.9	24	10	0.1566	10.35
4.6	18	5	0.6184	35.67
4.6	24	10	0.6118	43.95
NO_2				
10.6	0	5	0	0
16.2	0	5	0.0482	3.14
15.4	0	10	0	0
15.4	24	10	0.0809	6.14
25.7	0	10	0.0262	1.75
25.7	24	10	0.0209	1.39
26.9	0	10	0	0
26.9	24	10	0.1047	6.93
30.1	0	10	0.2333	15.28
30.1	24	10	0.3106	20.35
15.7	0	10	0.07882	4.85
15.7	24	10	0	0
46.1	0	10	0.3645	22.87
46.1	24	10	0.2760	17.32
30.0	0	10	0.1659	10.45
30.0	24	10	0.0898	5.67

TABLE 44. EFFECT OF A SINGLE 4-HOUR EXPOSURE TO MIXTURES OF O_3 AND NO_2 ON RAT LUNG WEIGHTS

Gas Conc. ppm		Postexposure Time to Sacrifice, hrs.	N	Lung Weight Difference From Controls	
				Grams	Percent of Control Lung Wt.
Mixture					
NO_2	O_3				
15.3	2.5	0	10	0.1878	12.55
15.3	2.5	24	10	0.1289	8.65
15.3	2.5	0	10	0.2789	25.49
15.3	2.5	24	10	0.2590	23.59
7.5	1.25	0	10	0	0
7.5	1.25	24	10	0.0626	4.34
15.5	1.25	0	10	0	0
15.5	1.25	24	10	0	0
7.4	2.5	0	10	0.1070	6.98
7.4	2.5	24	10	0.2053	13.40
27.3	1.7	0	10	0.2380	15.05
27.3	1.7	24	10	0.0888	5.61

Examination of Table 43 reveals that there is a fairly well defined effect threshold for O_3 somewhere between 1.25 and 1.9 ppm. However, this does not appear to be the case for NO_2 since the reproducibility of lung weight increase due to exposure is not as good as for O_3. This makes it difficult to analyze the mixture data in Table 44 on the basis of threshold effect, except for the conclusion that there is no obvious synergism. Apparently, exposure to O_3 alone caused an edematous response which maximized 24 hours after exposure, while NO_2 lung weight values 24 hours after exposure differed little from those obtained immediately postexposure. Most of the

time, exposure to the mixture led to maximum lung weights immediately after exposure. Although the experiment was designed as an examination of threshold effects, the data in the tables indicate that there is a relationship between concentration and lung weight increase. When the maximum percent lung weight increase obtained after any O_3 exposure is plotted vs. concentration, as in Figure 22A, a very good linear relationship is noted. The data are more scattered in the case of NO_2 (Figure 22B), but here too concentration and lung weight increases appear to be linearly related. If the concentrations of O_3 and NO_2 which cause 20% lung weight increase are compared, it is found that 37 ppm NO_2 is equivalent to 2.87 ppm O_3 and 12.9 ppm NO_2 to 1 ppm O_3. If the NO_2 line is transposed under this relationship, intersecting at 2.87 ppm O_3, the dashed line in Figure 22A is obtained. Considering the scatter of the NO_2 data, this line is not significantly difference from the O_3 line. When the slopes of the O_3 line and the transposed NO_2 are averaged, the line shown in Figure 23 is obtained. Points from pure O_3 exposures and from pure NO_2 exposures plotted as equivalent O_3 concentrations (1 ppm O_3 = 12.9 ppm NO_2) should lie about this line. If O_3 and NO_2 are additive with respect to edema formation, mixture points, plotted as equivalent O_3 concentrations, should also lie about this line. In Figure 23 the equivalent O_3 concentrations calculated from the mixture values in Table 44 are plotted against the maximum lung

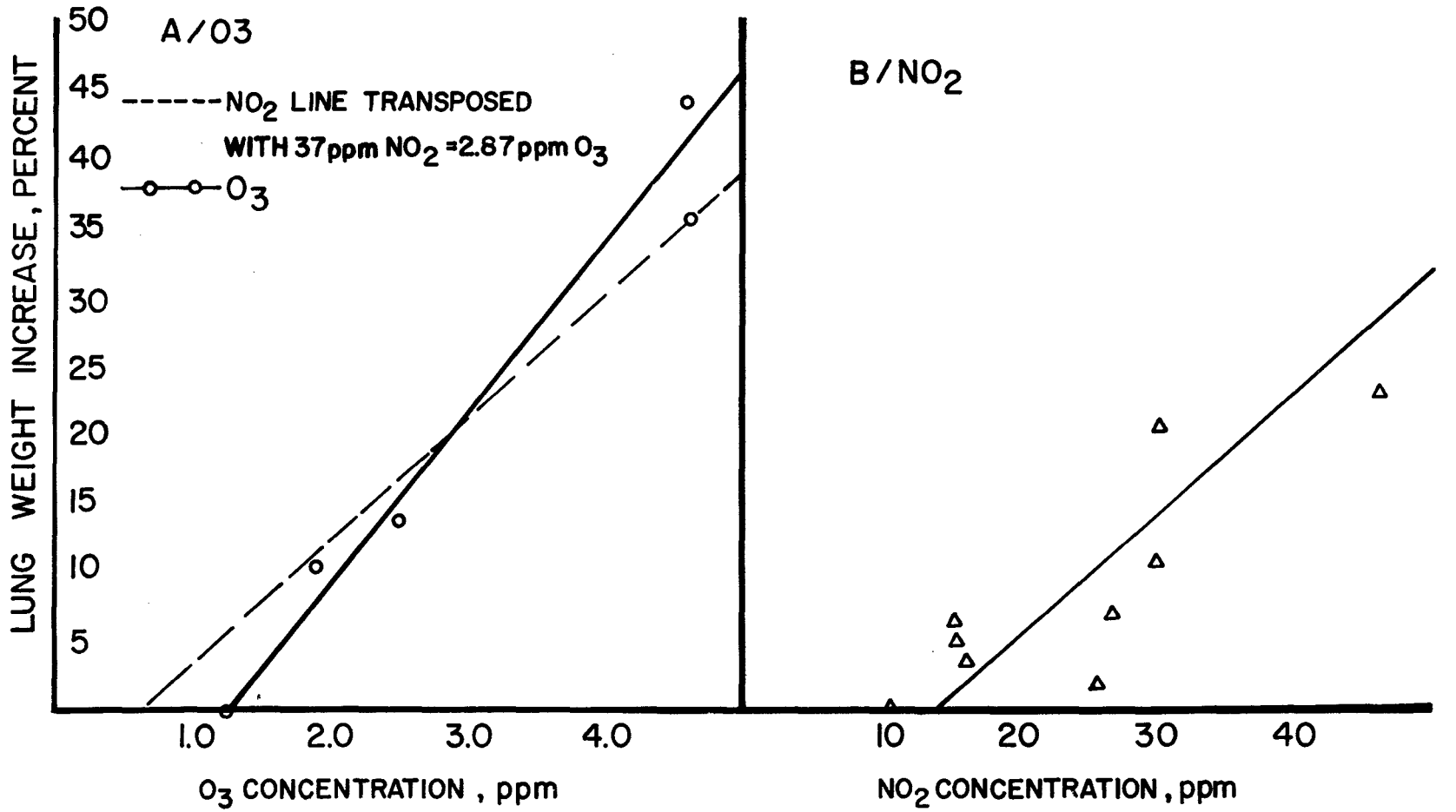


Figure 22. Effect of O_3 or NO_2 concentrations on rat lung weight.

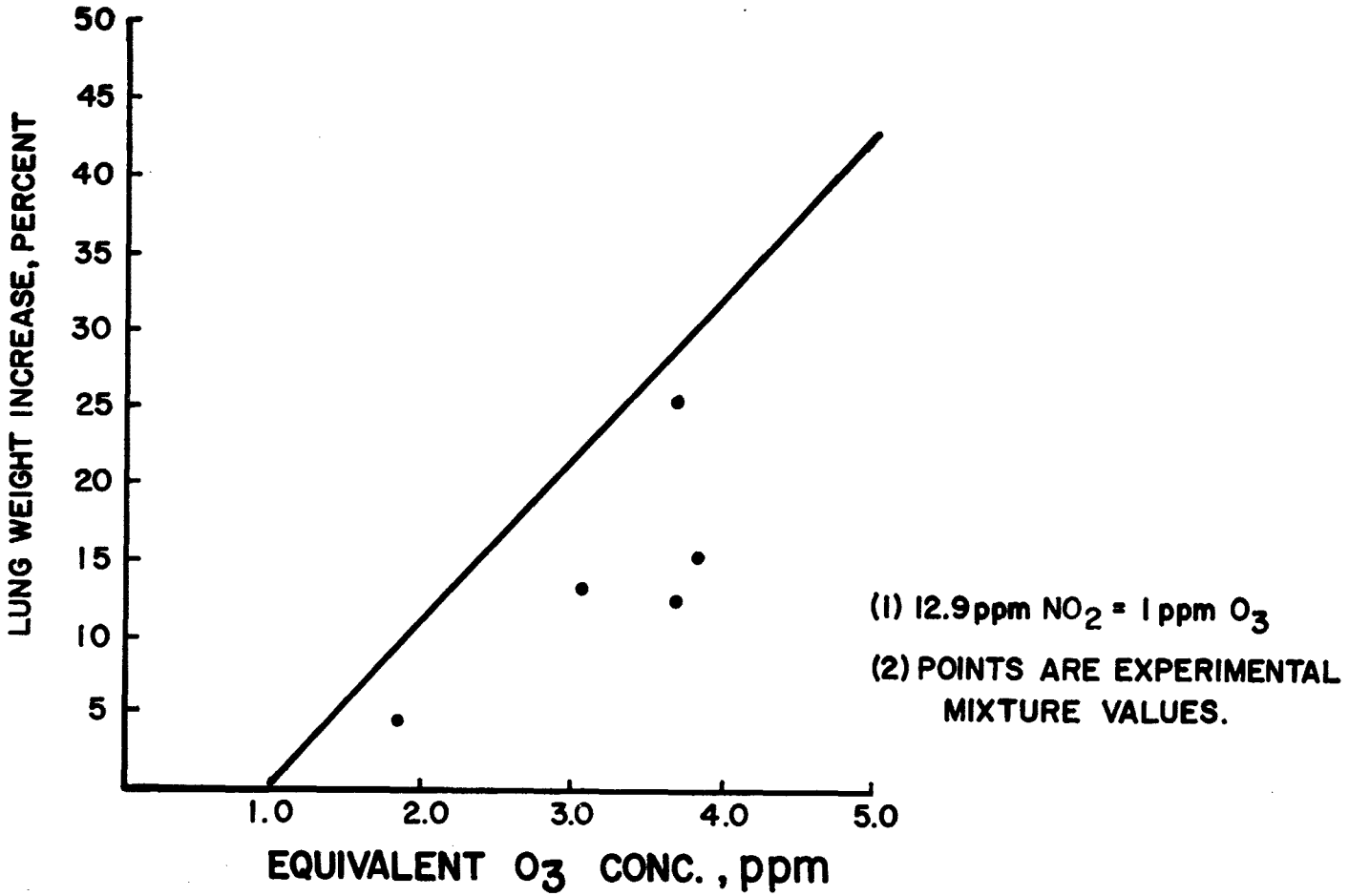


Figure 23. Relationship between effective mixture concentration and lung weight, assuming additivity of O_3 and NO_2.

weight increases obtained after exposure. All these points lie below the average line and, in fact, below both O_3 and transposed NO_2 lines. This implies that there is no synergism between O_3 and NO_2 with respect to edema production and that there may be some antagonism.

It is possible that any antagonism between the 2 toxic gases may result from their reaction:

$$2NO_2 + O_3 \longrightarrow N_2O_5 + O_2$$

Obviously, the contaminants were reacting in the chambers during exposures since it was necessary to introduce amounts of O_3 and NO_2 which would have given concentrations of 10 and 45 ppm individually in order to obtain 1.7 and 27 ppm in the mixture. Since N_2O_5 does not appear to have significant edemagenic effects at the concentrations generated, the effect is one of removal of the toxic components. Possibly that the reaction between O_3 and NO_2 continues in the airways leading to the alveoli and decreases their deep lung concentrations below those measured in the chamber. However, as the concentrations of the reactant gases O_3 and NO_2 are decreased to ambient pollution levels (0.3 ppm and below), the rate of the reaction will decrease and antagonism due to disappearance of the toxic gases will lessen; therefore, one might expect the interaction of O_3 and NO_2 to appraoch additivity at ambient levels.

Engineering Programs

The basic objectives of the Facility Engineering Department continue to involve administration of preventive maintenance programs and providing support to all phases of THRU operations. Efforts continue in updating existing equipment and procedures and increasing the effectiveness of all engineering procedures.

Six of the Thomas Dome animal exposure chambers were modified during the current report period to accommodate the special animal loading required for oncogenic studies of hydrazine compounds. These modifications required removal of dog pens from one of each pair of chambers used for exposure to one UDMH concentration and installation of racks and systems for exposure of large numbers of rats, mice and hamsters. To provide sufficient caging for the numbers of animals to be used, it was necessary to fabricate additional cages of a design similar to that used in Longley exposure chambers. These cages shown in Figure 24 have removable food baskets for rapid replacement of old feed which may have absorbed the exposure contaminant with fresh feed at the end of each intermittent exposure.

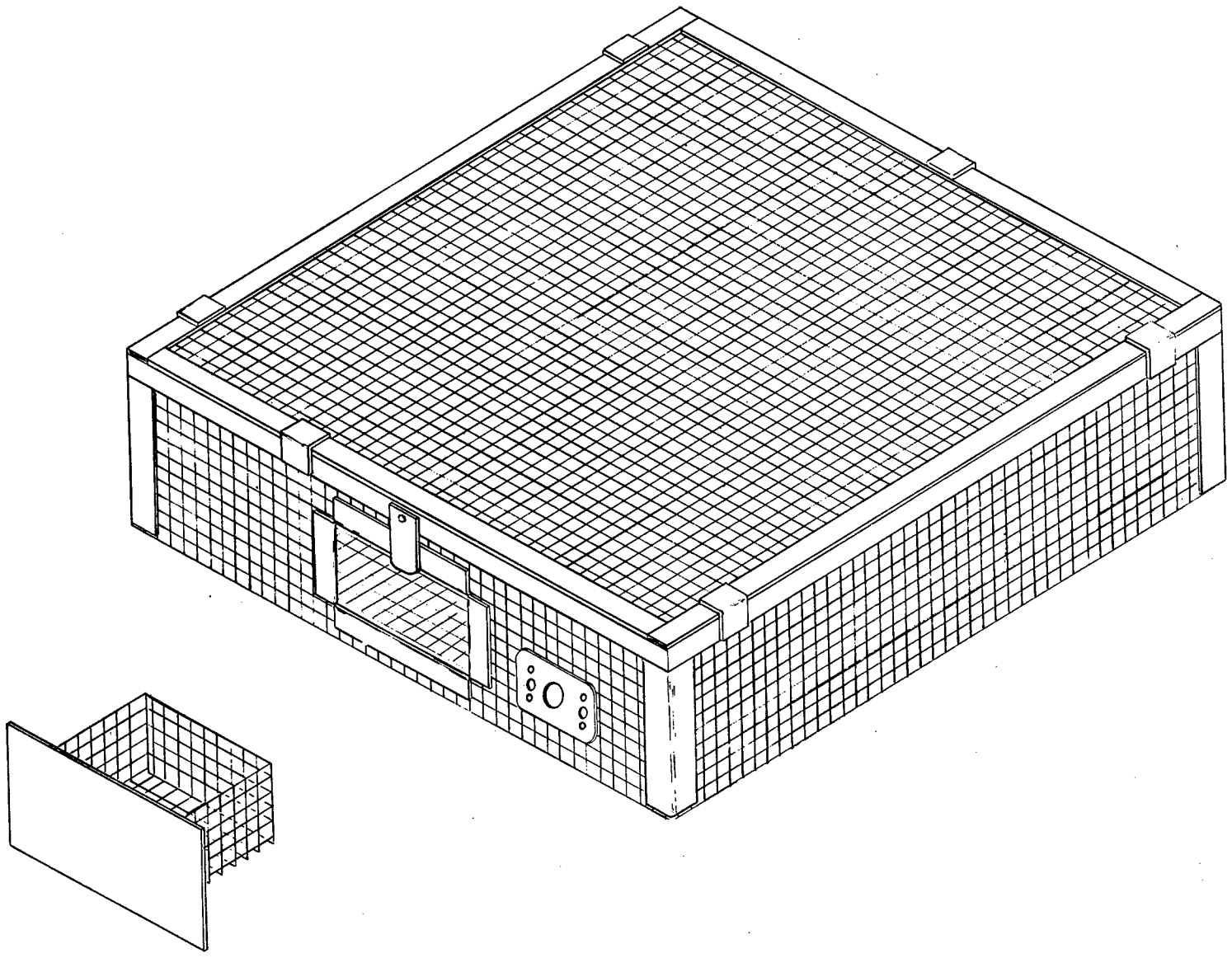


Figure 24. Animal cage style used for exposure of rodents to hydrazines.

A study of methods for more effective cleaning of wastes from cage pans was conducted and a suitable inexpensive disposable plastic tray was found that has been substituted for the stainless steel pans previously used. These disposable plastic trays are the type used for commercial packaging of meats. Their use has alleviated chamber cleaning problems and reduced the burden on our cage washing facilities.

Animal Weighing System - Computer Interface

The existing Animal Weighing System includes load cells of 0-10 pound and 0-50 pound ranges at each weighing station connected to a centrally located readout console. Dual readout units are provided which have a 4-digit capacity. Each unit has a separate binary-coded decimal (BCD) output which may be used for digital interfacing.

Operation of the system is as follows:

a. Animal identification number is given to console operator.

b. Animal is placed on weighing platform.

c. Console operator records animal identification number.

d. Console operator records animal weight.

e. Procedure is repeated for all animals in study.

f. Manually recorded information is submitted to statistics personnel to prepare punched cards.

g. Punched cards are used for computer input of data.

Several steps of the weight procedure from initial weighing to computer input were to be modified to reduce the time expended and to increase the reliability of the weight data. A system was designed to accomplish this by utilizing the digital output from the weight readout devices as shown in Figure 25. Manual operations affecting the recording of weight data were minimized significantly.

Operational steps of the modified system will be as follows:

a. Animal identification number is given to console operator.

b. Animal is placed on weighing platform.

c. Animal identification badge is placed in badge reader.

d. Badge reader initiates weighing cycle which consists of the following steps:

    1. Weight readout device indicates weight.

    2. Digital signal is transmitted to teletype unit.

    3. Hard copy and punched paper tape are prepared by the teletype.

    4. The system is reset for the next animal weight.

e. Steps "a" through "d" are repeated for each animal weight.

f. Punched paper tape and hard copy are removed from teletype.

g. Materials may be submitted directly for computer input or optionally may be verified before submittal.

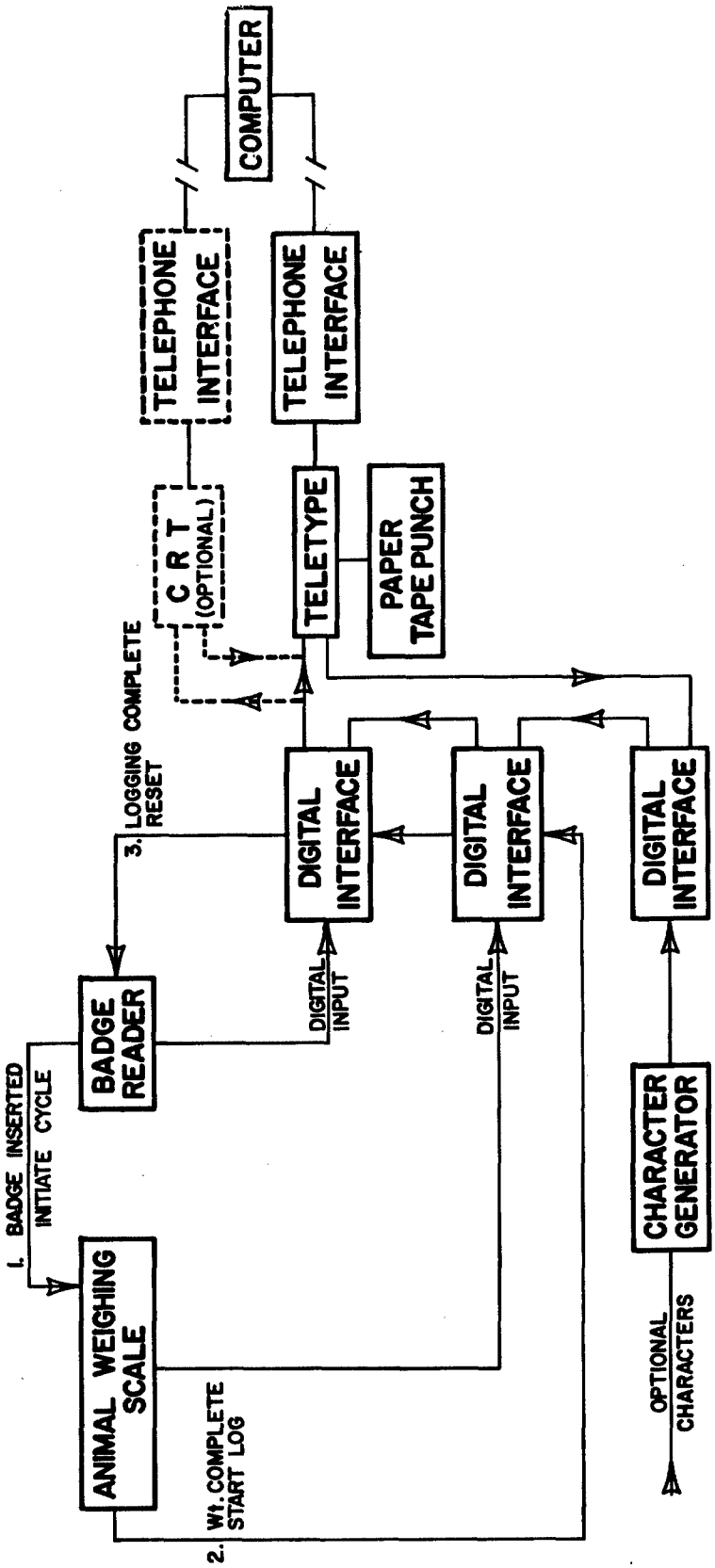


Figure 25. Schematic drawing of computer connected animal weighing system.

The digital interface system substitutes automatic assimilation of data for the following manual steps: recording of animal identification; recording of animal weights; and the transfer of animal weights to punched cards for computer input. These modifications provide for a reduction in weighing time and a reduction of possible weight data errors.

The operating components of the system are a parallel BCD to American Standard Computer Information Interchange (ASCII) code serial converter; an ASCII character generator, a BCD output badge reader, an ASR-33 Teletype terminal and an acoustic coupler modem.

The primary interface with the existing animal weighing system is at the animal weight readout device and the output of the animal identification badge reader. Output from these devices is a parallel BCD signal. This signal is converted to serial ASCII information by two 10 digit converters connected in series. These devices provide twenty characters of information consisting of animal identification and animal weights. An additional converter was constructed to provide compatible control signals required by the computer.

Operation of the system for animal weighing provides paper tape information according to the format shown in Figure 26 which is subsequently transmitted to the computer for programmed calculation.

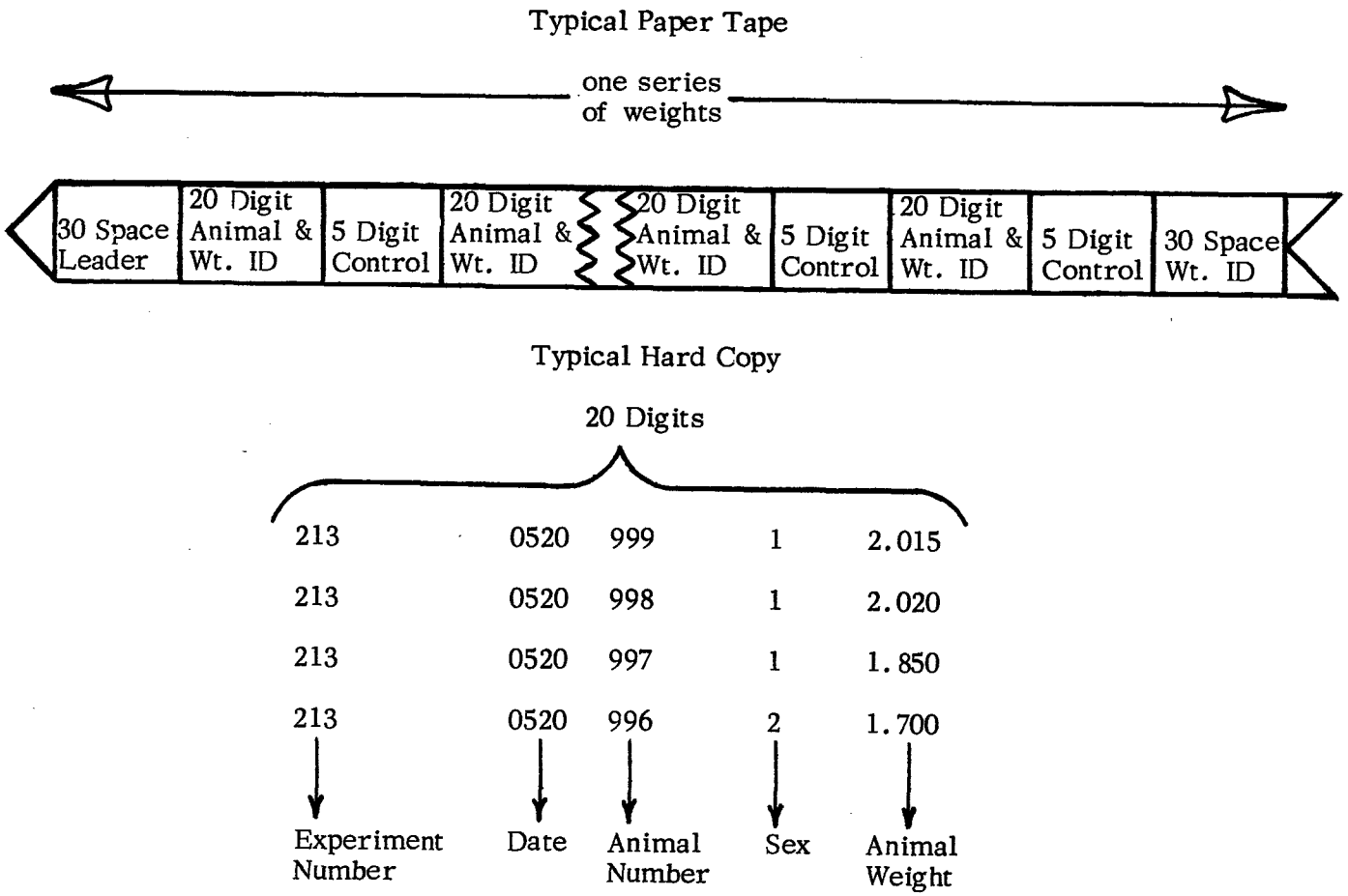


Figure 26. Example of paper tape code and printed copy of animal weight data.

The system provides additional processing capabilities for any systems in the facility which have digital outputs so that signals may be converted to hard copy or paper tape or transmitted to the computer by telephone line for storage or processing. The terminal component of the system is also available if desired for interactive processing with local or distant computers for tasks that may be computer oriented.

New Exposure Chambers

A scaled down version of the standard Rochester type inhalation exposure chamber was designed with an internal volume of one cubic meter. Two of these chambers as shown in Figure 27 were procured and installed in the THRU laboratory to increase the capabilities for conducting inhalation toxicity studies. The layout of the ambient laboratory with the new chambers installed is shown in Figure 28. Four additional chambers of the same type have been installed in the laboratories of the UCI Department of Community and Environmental Medicine's Sulfate Nitrate Inhalation Facility (SNIF) located in Irvine, California.

Noise Reduction Programs

Over the past several years, the THRU laboratory designated as Thomas Dome Room A has qualitatively appeared to become progressively noisier, a condition which represents a potential safety hazard and

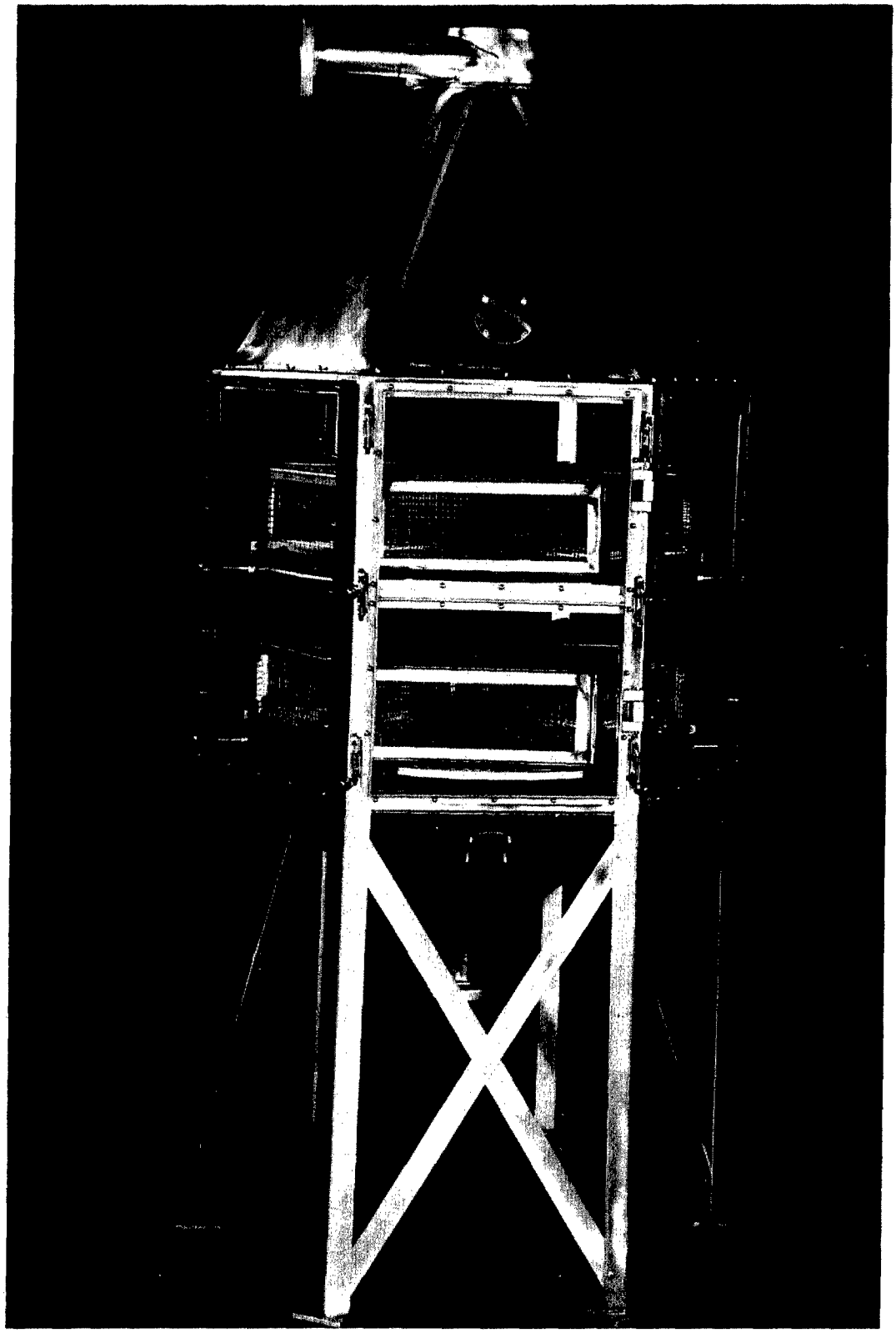


Figure 27. Rochester inhalation exposure chamber.

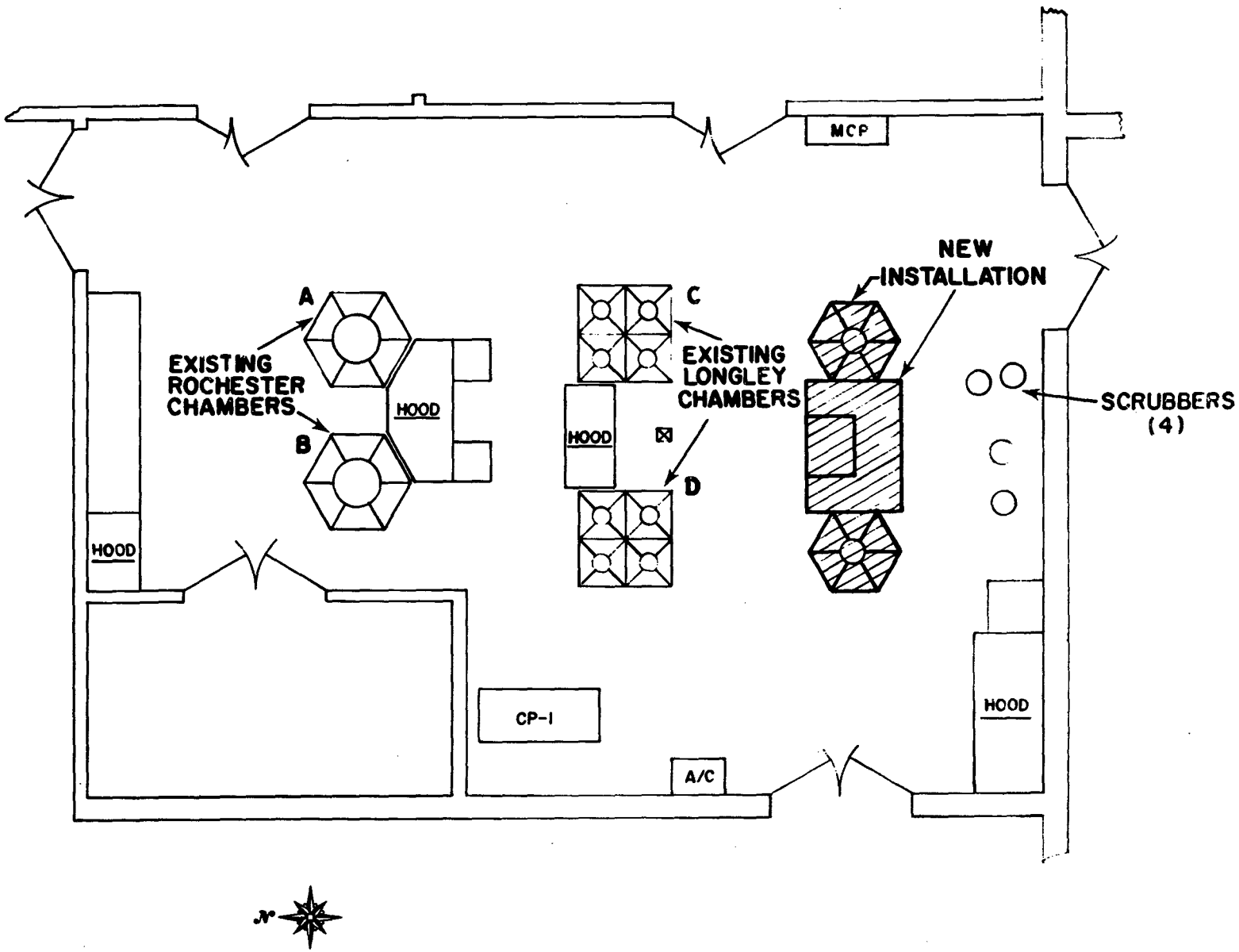
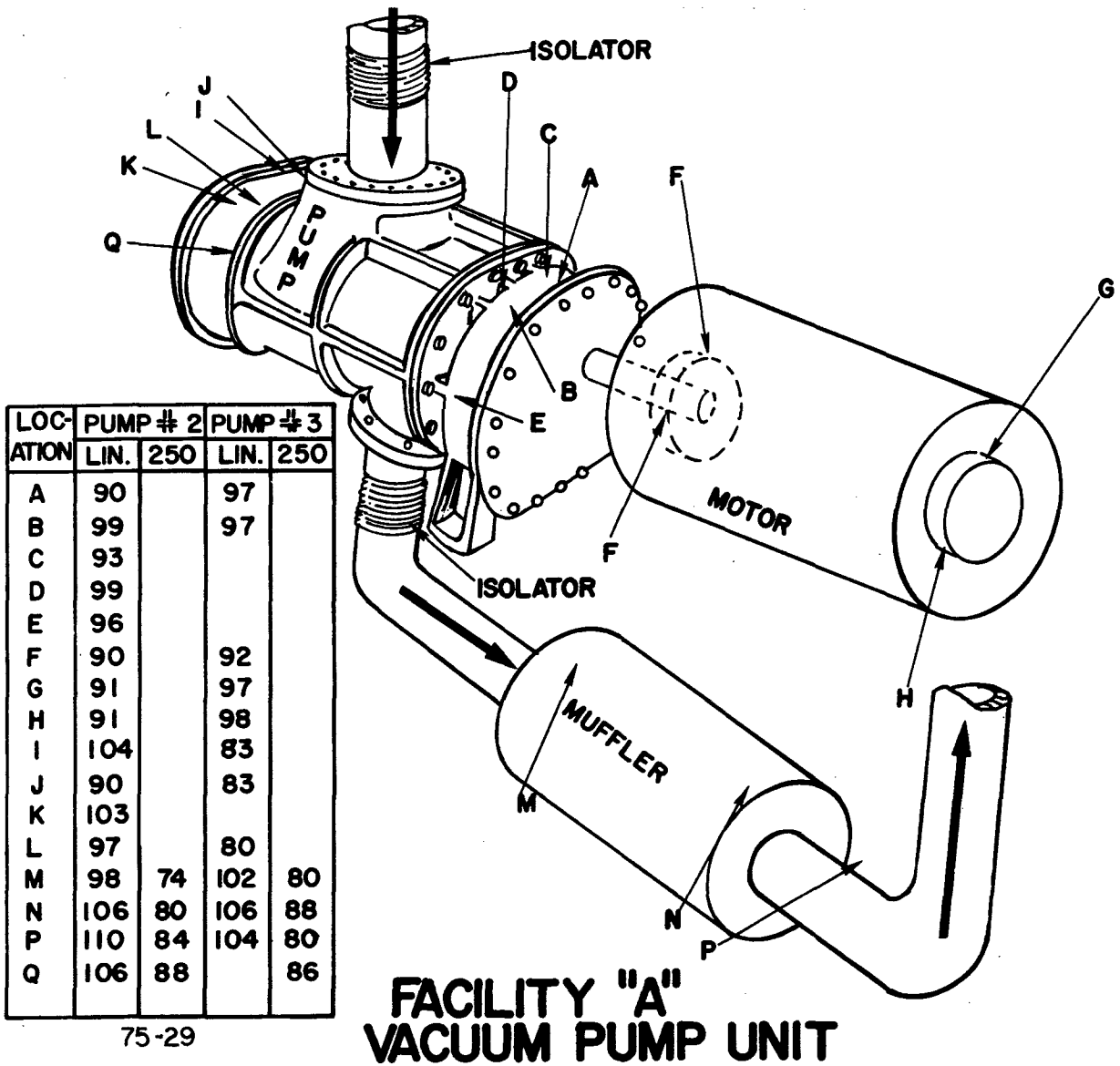


Figure 28. Layout of exposure chamber in the THRU ambient laboratory.

a voice communication problem. As a consequence of our safety program, an engineering study was conducted to determine the causes of the high noise levels and to identify potential methods for reduction of the noise to satisfactory levels. Noise levels were measured at a series of stations in the dome room and in the vacuum pump room located in the basement below. Measurements were initially made in the summer of 1974 and repeated under various operating conditions during the spring of 1975. The variations in operating conditions consisted of using different vacuum pumps alone and in combinations.

Without any vacuum pumps running, the background mechanical equipment noise ranged from 70-80 db in various parts of the room. Two of the pumps did not significantly alter the background level of noise but one pump raised the noise level from 2 to 14 db at various locations within the laboratory.

Analysis of the noise at the pumps using an acceleration probe showed sound pressure levels above 100 db at a resonant frequency of 500 Hz at various bearings as shown in Figure 29. These studies also indicated the muffler units on the vacuum pumps were not functioning properly.



LOC-ATION	PUMP # 2		PUMP # 3	
	LIN.	250	LIN.	250
A	90		97	
B	99		97	
C	93			
D	99			
E	96			
F	90		92	
G	91		97	
H	91		98	
I	104		83	
J	90		83	
K	103			
L	97		80	
M	98	74	102	80
N	106	80	106	88
P	110	84	104	80
Q	106	88		86

75-29

Figure 29. Points of noise measurement on chamber vacuum pumps.

A series of steps are being undertaken to eliminate the cause of the more significant noise sources identified. Among these are replacement of vacuum pump bearings, a more frequent preventive maintenance schedule, and repair or replacement of the mufflers. Vibration isolators will be installed in the pump exhaust pipe past the common manifold. The exhaust pipe passing through the dome room may also be insulated. Other actions will include insulation of hard surfaces at the dome room and sealing of partitions between the dome room and basement stairwell along with the installation of sound absorption material on these panels.

Training Programs

Chamber Technicians

Since no new chamber technicians were hired during this reporting period, all training programs were used as refresher courses for experienced technicians. The only change in Standard Operating Procedures was in the operation of the waste tank. Each technician has been instructed in this new procedure.

Written, deliberate and simulated Monthly Emergency Training Procedure tests were given to all chamber technicians during the year. These tests were given to insure maximum knowledge of what actions to take in case of a real emergency. The following list details the emergency training procedures covered during the past 12 months:

Date	Procedure	Personnel Participation*
June 1974	Vacuum Pump Failure	A
July 1974	Complete Power Failure	A
Aug. 1974	Air Compressor Failure	A
Sept. 1974	Supply Air Fan	A
Oct. 1974	Fire in Dome During Entry	A, B, C
Nov. 1974	Air Compressor Failure	A
Dec. 1974	Fire in Air Lock During Entry	A, B, C
Jan. 1975	Rescue of Incapacitated Dome Entrant	A, B, C
Feb. 1975	Vacuum Pump Failure	All chamber technicians
March 1975	Air Compressor Failure	All chamber technicians
April 1975	Complete Power Failure	A
May 1975	Air Supply Fan Failure	A

*A - Shift Operator
B - Safety Observer B
C - Safety Observer C (used only during altitude experiments)
D - Dome Entrant

Due to the loss of several experienced personnel, it has been necessary to train all remaining chamber technicians to draw blood samples from various laboratory animals. This program has been most successful and now most chamber technicians can draw blood samples from dogs, monkeys, rabbits, rats and hamsters. This training along with the Aerospace Medicine course in November, 1974 has resulted in a better educated and more versatile group of chamber technicians taking care of animals. All chamber technicians have had much practice in handling all species of animals. They have also been trained in the operation of the weighing console for weighing animals in the domes.

Five chamber technicians took the AALAS tests in May, four at the first level, and one at the second.

A few of the chamber technicians have worked in the Ambient Laboratory on studies there. They have had the opportunity to learn about other areas in toxicology through this practical training.

Due to the large number of rodents being held at this facility, a new recordkeeping system had to be devised. A new system of mortality ledgers and weekly checks has been developed. This system makes keeping track of animal deaths and the location of rodents much easier. A daily ratio of mortalities for each experiment is now available.

A training class was given in March on the use and operation of the Scott Air Pak. All day shift and weekend personnel now know how to use it.

Animal Technicians

Animal Care Training Programs described in last year's annual report were continued this year as programmed.

All incoming technicians are required to complete the Ralston Purina Animal Care Course. This course is primarily a self study course which lays a foundation for further study in the field of Laboratory Animal Science. Six technicians successfully completed this course during this reporting period.

Since last year's annual report, one technician has been certified at the highest level (laboratory animal technologist) and one began employment last October. There are several technicians who desire to become certified in the AALAS program, but must fulfill the one year time and experience requirement before taking the examination for the first level of certification (assistant animal technician). It is expected that at least 8 technicians will become certified as assistant animal technicians and 2 as animal technicians.

The basic course outline for certification by AALAS is as follows:

Assistant Laboratory Animal Technician

	(Suggested weighted importance of subjects)	
I.	Introduction	5%
II.	Basic Structure and Function of Body Systems	5%
III.	Nutrition	5%
IV.	Genetics and Mating Systems	5%
V.	Animal Handling, Restraint, and Identification	30%
VI.	Equipment and Materials: Identification, Maintenance and Proper Use	20%
VII.	Sanitation and Hygiene	10%
VIII.	Animal Health and Disease	10%

IX.	Animal Nursing	5%
X.	Animal Experimental Techniques	5%
		100%

Laboratory Animal Technician

(Suggested weighted importance of subjects)

I.	Introduction	5%
II.	Anatomy, Physiology, and Related Disorders	10%
III.	Nutrition and Metabolism	5%
IV.	Genetics and Mating Systems	5%
V.	Physiological Parameters, Breeds, Strains, and Behavioral Traits	10%
VI.	Veterinary Pharmacology, Anesthesia, Euthanasia	10%
VII.	Animal Health and Disease	10%
VIII.	Housing and Equipment Design	10%
IX.	Sanitation, Hygiene, and Safety	5%
X.	Administration, Management, and Record Keeping	5%
XI.	Shipping and Receiving Animals	5%
XII.	Gnotobiology and Germfree Animal Techniques	5%
XIII.	Animal Experimentation Techniques	5%
		100%

Laboratory Animal Technologist

		(Suggested weighted importance of subjects)		Total Group %
I.	A.	Introductory Studies and Regulations	3%	11%
	B.	Basic Concepts in the Sciences	3%	
	C.	Comparative Anatomy, Physiology, and Related Disorders	5%	
II.	A.	Administration, Management and Record Keeping	10%	25%
	B.	Housing and Equipment Design	5.5%	
	C.	Shipping and Receiving Animals	4.5%	
	D.	Sanitation, Hygiene, and Safety	5%	
III.	A.	Nutrition and Metabolism	4%	18%
	B.	Genetics and Mating Systems	5%	
	C.	Physiological Parameters, Breeds, Strains, and Behavior Traits	5%	
	D.	Handling and Identification	4%	
IV.	A.	Animal Experimental Techniques	4.5%	11%
	B.	Gnotobiology and Germfree Animal Techniques	3%	
	C.	Radiology	3.5%	
V.	A.	Animal Health and Disease	6%	20%
	B.	Veterinary Pharmacology	4.5%	

				Total Group %
	C.	Veterinary Anesthesia and Euthanasia	4.5%	
	D.	Surgical Nursing	5%	
VI.	A.	Veterinary Clinical Pathology: Parasitology	3%	15%
	B.	Veterinary Clinical Pathology: Hematology	3%	
	C.	Veterinary Clinical Pathology: Urinalysis	3%	
	D.	Veterinary Clinical Pathology: Mycology	3%	
	E.	Veterinary Clinical Pathology: Bacteriology	3%	
				100%

The need for a formal course in laboratory animal science was evident after conducting 5 coal tar aerosol experiments over a 3-year period which began in 1972. All animals in these aerosol experiments are held for their lifetime after the exposure phase of the experiment. The THRU technicians must now be able to identify various animal diseases as well as skin tumors in our colony of approximately 3000 animals currently being housed at the THRU. Current trends in the conduct of chronic inhalation

studies suggest the need for long-term postexposure observation and testing of experimental animals. Therefore, education of the entire group of technicians in the field of laboratory animal science is of great concern particularly as it relates to animal care and maintenance.

The School of Aerospace Medicine at Brooks Air Force Base has written, distributed and conducted formal classroom training as an aid to all technicians at the THRU and particularly those technicians desiring to take the AALAS certification examinations. The course consists of 47 hours of lecture and related laboratory procedures in the field of laboratory animal science. Video tapes of the course were made by THRU for future training. The following list details the training procedures covered by the course. The chamber technician group was also included in this course of study.

Subject	Number of Hours
Introduction of Anatomy and Physiology	1
Skeletal System	2
Muscular System	3
Central Nervous System	4
Respiratory System	5
Cardiovascular	6
Cardiovascular System and Urinary System	7
Reproductive System	8

Subject	Number of Hours
Digestive System	9-10
Endocrine System	11
Skin and Appendages	12
Fundamentals of Disease	13-15
Metric System	16
Fundamentals of Disease	17-18
Animal Welfare Act	19
Primatology	20
Pharmacology	21-24
Clinical Laboratory	25
Parasitology	26-27
Procurement and Quarantine	28
Standardization	29
Records, Identification and Gnotobiology	30
Nutrition	31
Primate Diseases	32-33
Dog Diseases	34
Cat Diseases	35
Sanitary Standards	36
Rabbit and Rodent Diseases	37-39

Subject	Number of Hours
Zooneses	40-42
Technician Certification and Closing Remarks	43
Anatomy and Physiology Cat Dissection Laboratory	44-47

After the completion of the course work, the Air Force instructors distributed a practical training work sheet that each technician should complete as time and opportunity becomes available.

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